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50th Anniversary Annual Meeting & ToxExpo™

The Official Journal of the Society of Toxicology

TOXICOLOGICAL SCIENCES

Washington, D.C.
March 6–10, 2011
Walter E. Washington Convention Center

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Deadline for Proposals for SOT 2011 Annual Meeting Sessions: April 30, 2010

WHY SUBMIT A PROPOSAL?

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

SESSION TYPES

Continuing Education—Emphasis on quality presentations of generally accepted, established knowledge in toxicology
Note: CE Courses will be held on Sunday.
Symposia—Cutting-edge science; new areas, concepts, or data
Workshops—State-of-the-art knowledge in toxicology
Roundtables—Controversial subjects
Historical Highlights—Review of a historical body of science that has impacted toxicology
Informational Sessions—Scientific planning or membership development
Education-Career Development Sessions—Sessions that provide the tools and resources to toxicologists that will enhance their professional and scientific development

2011 Thematic Approach

The Scientific Program Committee will continue the thematic approach for the 2011 Annual Meeting. All proposal submissions will be reviewed for their relevance under the following themes for the 2011 meeting:

• Global Air Quality and Human Health
• Novel Approaches to Preclinical Safety Assessment: Bridging the Gap between Discovery and the Clinic through Translational Toxicology
• Environment and Disease
• Toxicity Testing: State of Science and Strategies to Improve Public Health
• Integration of Toxicological and Epidemiological Evidence to Understand Human Risk
• Emerging Global Public Health Issues

Please note that while we are actively soliciting proposals for the themes listed above, all proposal submissions will be reviewed under the current criteria for their timeliness and relevance to the field of toxicology.

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 49th Annual Meeting of the Society of Toxicology, held at the Salt Palace Convention Center, March 7–11, 2010.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 473.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 496.

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

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BIOLOGICAL PATHWAY ANALYSIS: AN INTRODUCTION TO THE PATHWAY KNOWLEDGE BASES FOR TOXICOLOGICAL RESEARCH.

M. E. Gillespie, Department of Pharmaceutical Sciences, St. John’s University, Queens, NY.

Genomic and proteomic datasets are a complex but information rich resource. ToxicoLOGY is expanding to new omics-based technologies to identify important gene and protein expression changes. A critical step in such studies is the analysis of the data set to derive reasonable mechanistic meaning and testable hypothesis. Additionally, the use of genomic and proteomic approaches to identify new lead molecules for biologically relevant targets is rapidly expanding. A challenge for scientists is how to properly and effectively incorporate high-throughput omics technologies into their research programs. This course will present practical cases demonstrating how the Reactome pathway analysis tools can be used to identify relevant biological pathways within large and immensely complex data sets derived from multiple high-throughput technology platforms. The course will begin with an overview of how genomic and proteomic approaches to identify new lead gene and protein expression changes. A critical step in such studies is the analysis of the data set to derive reasonable mechanistic meaning and testable hypothesis. Often the analysis of interaction data, and RNAi screening. All of these methods share a common end-point, the generation of large datasets that the toxicologist must analyze without prior knowledge of a reasonable mechanistic basis or outcome. The analysis of the data must take into account both positive and negative interactions, and not limited to, microarray gene expression data, mass-spectrometry data, protein interaction data, and RNAi screening. All of these methods of analysis should be considered collectively when designing the battery of nonclinical safety studies. Unique considerations for each of these product classes will be highlighted.

BIOLGICALS: INTRODUCTION TO DRUG DEVELOPMENT.

J. D. Green1 and L. Andrews2. 1Biogen Idec, Inc., Cambridge, MA and 2Genzyme Corporation, Framingham, MA.

Toxicologists and other preclinical scientists have developed an extensive experience base with a wide range of preclinical drugs. The development of new knowledge bases, often called pathway databases, incorporates information on protein, gene, and literature databases to facilitate the identification of relevant schemes using combinations of data, resulting in predictions that more closely approximate biological networks. The course will review how available knowledge bases such as Reactome and PharmGKB can be used to interrogate large and complex datasets to identify the contributions of specific pathways in a given biological response to toxicant exposure.

COMPARATIVE BIOLOGY OF THE LUNG.

R. Parent1 and D. Costa2. 1Consultox Ltd., Damariscotta, ME and 2U.S. EPA, Research Triangle Park, NC.

All mammals have evolved respiratory structures to ensure that the principal function of the lung, gas exchange, is met under varying physiological conditions. However, these essential functions are achieved despite significant differences in the structural organization, cellular composition, and related functions mediated through the respiratory system and across mammalian species. Translational toxicology requires that one understand these innate differences in fundamental respiratory biology if one is to appropriately interpret and extrapolate findings in animal models. On a gross level, the nasal passages, pleural thickness, vascularization, and connective tissue structure vary between species. Quantitative evaluation of the tracheobronchial airway tree demonstrates few consistent features between species. The epithelial cell populations lining the lung differ in cell type, location, and abundance. The metabolic enzymes, cytokines, chemokines, protease, and anti-oxidant potential, although showing some similarities, also demonstrate vast differences. Similarly, basic immunological functions in laboratory animals must be understood and related to those in humans to enable appropriate species translation. We will illustrate many of these fundamental differences, describe methods for making measurements in different species, and most importantly, focus on the fundamentals of appropriate interpretation of study data derived in animals for human use. Attendees will gain a basic understanding of the value and pitfalls extending from these species differences, which will enable improved study design and extrapolation of research data for efficacy, safety pharmacology, and toxicology studies. This course is intended to provide attendees with a basic understanding of lung structure-function relationships and associated immunological and metabolic functions in laboratory animals that will aid in the extrapolation of inhalation or respiratory data to humans.

CYTOKINES: BALANCING THERAPEUTIC UTILITY AND IMMUNE SYSTEM-MEDIATED.

L. A. LeSauter1 and R. A. Ponce2. 1Preclinical Sciences, Immunology, Charles River, Montreal, QC, Canada and 2Preclinical Development, Amgen, Inc., Seattle, WA.

Direct and indirect modulation of cytokines via therapeutics, either increasing or decreasing cytokines, is a central factor in the success of current therapies targeting cancer, autoimmunity, inflammation, and infection. Nonclinical and clinical data demonstrate that these therapies can overwhelm compensatory mechanisms designed to protect the host, resulting in toxicity. The therapeutic benefits and potential toxicities can be best understood through an understanding of the central role of cytokines in modulating cellular function. To address these specific issues, we will define the central toxicities and syndromes that have been identified as arising from cytokine-mediated immunomodulation; establish the immunological basis for these toxicities using in-depth exploration where possible, including useful biological markers that can inform clinicians and toxicologists; develop an understanding of cytokine modulation in the treatment of cancer, autoimmunity, inflammation, and infection; and identify deficiencies in current toxicological practice for predicting certain immune system-mediated risks arising from cytokine-mediated immunomodulation in humans. Finally, we will explore specific case studies where these principles have been applied to reinforce these central concepts.

NUCLEAR RECEPTORS: ROLE IN CHEMICAL MODE OF ACTION AND TARGETS FOR TOXICITY TESTING.

C. Corton1 and J. P. Vanden Heuvel2. 1Environmental Carcinogenesis Division, U.S. EPA, Research Triangle Park, NC and 2Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA.

Nuclear receptors (NR) are one of the most abundant classes of transcriptional regulators in animal and function as ligand-activated transcription factors. They provide a direct link between signaling molecules and transcriptional responses that impact diverse functions including development, metabolic homeostasis, and reproduction. NR are not only promising pharmacological targets but can be activated inappropriately by environmentally relevant chemicals leading to a broad spectrum of adverse effects. The intent of this basic course is to provide an overview of the biology of nuclear receptors, the pathways and modes of action of a subset of nuclear receptors involved in chemical toxicity, and strategies for screening chemicals for NR interactions as well as placement in mode-of-action categories. To begin with, we will cover the structure, function and general mechanisms of activation as well as basic biological roles of NR that are targets of xenobiotics in different tissues and cell types. We will then explore the role of NR in both augmenting and suppressing chemical carcinogenesis, which will include a summary of mode of action and human relevance of those NR (CAR, PPAR, PXR, RXR) commonly associated with liver cancer. Following this summary, the adverse effects of xenobiotics on the endocrine system associated with activation or modulation of estrogen, androgen, and thyroid hormone receptors will be addressed. Finally, both the primary and secondary screening strategies to define effects of chemicals on NRs and the pathways that mediate their adverse effects will conclude this course. The intended audience for this course includes those who desire a basic knowledge of the state of the science of nuclear receptors in chemical mode of action and strategies for accelerating the placement of chemicals into mode-of-action pathways. The course will be of interest to many who are engaged in wider aspects of carcinogenesis, reproductive biology and risk assessment.
PREDICTIVE POWER OF NOVEL TECHNOLOGIES (CELLS TO ‘OMIC’S): PROMISES, PITFALLS, AND POTENTIAL APPLICATIONS.

S. S. Nadadur and M. Cunningham, Division of Extramural Research and Training, NIEHS, Research Triangle Park, NC and 2 NanoMics Biosciences, Inc., Cary, NC.

Advances in the disciplines of cell and molecular biology have led to the development of novel biotechnologies capable of generating "global molecular profiles" for in situ toxicological assessment. These technologies should accelerate our understanding of the molecular basis for susceptibility to toxicants and provide new insights into mechanisms of action. Both theoretical and practical information on these emerging high-throughput technologies and their applicability, interpretation, and integration will present a more comprehensive understanding of cellular responses to chemical/toxicant stress. To begin, the course will highlight the utility of laser capture microdissection in isolating specific cell populations for toxicological assessment and the risks that pharmaceuticals, pesticides, and other toxic substances pose to the agent or after exposure of the eye.

Ocular toxicity is known to occur following different routes of exposure. The course will highlight the utility of laser capture microdissection in isolating specific cell populations for toxicological assessment of ocular toxicity. Slit lamp biomicroscopy and indirect ophthalmoscopy are routinely utilized to more closely evaluate the anterior and posterior chambers of the eye, respectively, during the course of toxicology studies. At the time of necropsy, ocular tissues are collected and processed for histopathological evaluation. More specialized endpoints, such as electrophotography, can be incorporated, as needed. Ocular anatomy and physiology and the assessment of ocular toxicity can be challenging to scientists involved in the safety assessment of pharmaceuticals, pesticides, and other agents. This basic course will cover ocular anatomy and physiology in laboratory animals, established methods used to assess ocular toxicity, as well as more novel techniques for toxicity assessment.

Examples of ocular toxicity that can occur following different routes of exposure will be discussed.

GENE-ENVIRONMENT INTERACTIONS INFLUENCE CYTOKINE BIOLOGY IN IMMUNOTOXICITY AND DISEASE: GENOMIC, GENETIC, AND EPIMICROSCOPIC PERSPECTIVES.

B. Yuceloglu and V. I. Johnson, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, CDC/NIOSH, Morgantown, WV.

Cytokines are key signaling and effector molecules that regulate many aspects of host response to exogenous stressors. To date, animal and human studies have identified individual and interacting effects of cytokines at different stages in the pathogenesis of chronic inflammatory and immune-mediated diseases. Animal studies utilizing gene knock-out and transgenic animals and expression microarrays have identified disease-related cytokine networks. Human studies using various genome screening efforts have also uncovered potential candidate genes for disease development and progression. Cytokine genes and their receptors are highly polymorphic and can undergo sequence variation in these genes have been associated with the course of and susceptibility to a variety of diseases including infectious, inflammatory, and autoimmune.

In addition, epigenetic changes including altered DNA methylation and histone acetylation can control cytokine gene expression by changing the transcription-permissive nature of chromatin structure. Environmental factors are known to modify the direction and magnitude of disease risk in an environment-specific manner. In this context, genome association and genome-wide association studies have identified interactions between cytokine gene variations and environmental/occupational exposures as shown in the case of silicosis and asthma. In addition, recent studies demonstrated that environmental exposures might alter methylation states of key cytokines genes supporting an epigenetic gene-environment interaction.

The course will address aspects of the current state of knowledge with respect to genomic, genetic, and epigenetic approaches in the investigation of cytokine genes associated with occupational and environmental-related diseases.

REPRODUCTION AND REGULATORY IMPACT.

R. E. Chapin and J. S. Moffet, 1 DART, Pfizer Global Research and Development, Groton, CT and 2 Beecherger Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.

Most new compounds destined for commerce, and all compounds intended for human consumption, need to be assessed for developmental and reproductive toxicity (DART). However, the underlying biology can be confusing because the jargon employed by the cognoscenti can be impenetrable and the implications of findings in these studies are often difficult to appreciate quickly. Our panel will begin this course with an open discussion about the value and limitations of these issues. After a quick review of some of the key biology, we will touch on the characteristic study designs which generate the necessary data. A point of focus will have the panel examine the typical effects seen in adults and juveniles, and what impact these can have on the registration and use of the compound in the U.S., respectively. Although the focal point for this course will be on environmental compounds, the final presentation will highlight drug candidates and how reproducitive or developmental findings affect their journey to the marketplace. It is our goal to leave students with a better understanding of the impacts that reproductive or developmental findings have on the registration and use of environmental and pharmacologic compounds.

ASSESSMENT OF OCULAR TOXICITY IN TOXICOLOGY STUDIES CONDUCTED FOR REGULATORY PURPOSES.

M. C. Collins and A. Welf, 1 Preclinical Services, Charles River Laboratories, Reno, NV and 2 Charles River Laboratories, Reno, NV.

Ocular toxicity is known to occur following intended or unintended exposure of ocular tissues to xenobiotics. It can occur following local exposure of the eye to an agent or after exposure via oral or other routes of administration. In order to define the risks that pharmaceuticals, pesticides, and other toxic substances pose to the eye, an assessment of ocular toxicity is routinely included in general toxicology studies conducted for regulatory purposes. Because anamnestic and physiological differences between species can impact the nature of the ocular effects observed, understanding species differences is important. Although it is possible to detect some ocular effects, such as conjunctivitis, with the naked eye, more sensitive techniques are routinely used to assess ocular toxicity. Slit lamp biomicroscopy and indirect ophthalmoscopy are routinely utilized to more closely evaluate the anterior and posterior chambers of the eye, respectively, during the course of toxicology studies. At the time of necropsy, ocular tissues are collected and processed for histopathological evaluation. More specialized endpoints, such as electrophotography, can be incorporated, as needed. Ocular anatomy and physiology and the assessment of ocular toxicity can be challenging to scientists involved in the safety assessment of pharmaceuticals, pesticides, and other agents. This basic course will cover ocular anatomy and physiology in laboratory animals, established methods used to assess ocular toxicity, as well as more novel techniques for toxicity assessment.

Examples of ocular toxicity that can occur following different routes of exposure will be discussed.

MITOCHONDRIAL TOXICITY: ANIMAL MODELS AND SCREENING METHODS IN DRUG DEVELOPMENT.

Y. Will and C. Palmeira, 1 Pfizer Global Research and Development, Groton, CT and 2 University of Coimbra, Coimbra, Portugal.

Mitochondria produce almost all the energy in cells, but also chronically expose the cell to cytotoxic free radicals. Mitochondrial disease and toxicity is a rapidly advancing field and the consequences of mitochondrial impairment should be appreciated by scientists in all disciplines. It is estimated that more than 75 diseases and metabolic disorders are attributable, at least in part, to mitochondrial dysfunction. Mitochondrial dysfunction can lead to many different pathologies of the liver, heart, muscle, kidney, and CNS through diverse mechanisms. Numerous widely prescribed therapeutics can undermine mitochondrial function by interfering with DNA replication or expression, and more acutely, by uncoupling or inhibiting oxidative phosphorylation, leading to organ dysfunction and damage. In addition, numerous environmental agents can contribute to diseases and toxicity through modifications of mitochondrial function, leading to examples for Parkinson's Disease and Autism. This course will review fundamental concepts of mitochondrial biology and the many different mechanisms by which xenobiotics interfere with mitochondrial function. Both common and novel in vitro screening approaches will be described, as well as in vivo animal models used to study mitochondrial-mediated toxicities and pathologies, with an emphasis on both their utility and limitations. The course will also introduce Structure-Activity Relationship and systems biology approaches to reveal common factors and novel mechanisms of mitochondrial toxicity. Upon completion of this course, participants will have a deeper understanding of how xenobiotics can alter the basic biochemistry and physiology of mitochondria, how minute changes in mitochondrial processes translate into complex toxicities, organ pathologies, and diseases, as well as a basic understanding of how to study mitochondria and mitochondrial dysfunction.
ICH INITIATIVES FOR CONDUCTING PHARMACEUTICAL PRECLINICAL SAFETY STUDIES: NEW AND REVISED GUIDELINES AND CHALLENGES.
T. Wang1 and D. W. McGuinn2, 1Preclinical Safety, Neuartis Pharmaceuticals Corporation, Emeryville, CA and 2Division of Drug Oncology Products, U.S. EPA, Silver Spring, MD.

In recent years, the International Conference of Harmonization (ICH) Expert Working Groups have been developing new guidelines and revising some of the existing guidelines on preclinical safety requirements. Some of the important recent initiatives include new guidance, ICH S9, for preclinical evaluation of anticancer pharmaceuticals, revision of ICH M3 guidance that addresses the timing of pre-clinical studies in relation to various stages of clinical development, and new guidelines on genotoxicity testing (ICH S2) that replaces and combines the ICH S2A and S2B guidelines. Over the past decade, substantial experience regarding preclinical safety evaluation of biologics (ICH S6) has been gained and based on this experience revision of S6 is underway. The latest rationale behind the new initiatives at ICH will be discussed, while a panel of experts will present new developments and key challenges in each of the areas mentioned above and will provide expert commentary and perspective on the potential impact on preclinical safety evaluation programs these guidelines may have. Case studies will be used to highlight detailed examples, experience in conducting non-clinical ICH safety studies, and the acceptance of the ICH guidelines by the practicing regulatory organizations and reviewers. Our panel experts have years of experience in preclinical toxicology testing from either an industry or regulatory perspective. In addition, several have represented the United States on the ICH Expert Working Groups, and participated in writing or revising these ICH guidelines. This panel will be available to answer questions that will allow participants to obtain valuable information on this topic.

SEGMENT-SPECIFIC RENAL PATHOLOGY FOR THE NON-PATHOLOGIST.
D. Hoivik1 and S. E. Meigh Hirt2, 1Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT and 2Drug Safety and Metabolism, Auxilium Pharmaceuticals, Inc., Malvern, PA.

The structural and functional complexity of the kidney uniquely predisposes it to be a sensitive target organ for a number of toxicants. By taking a segment-specific approach to the kidney, participants will gain a broad understanding of structure and function, changes, species and gender-related differences in renal structure and function, spontaneous changes, the utility of biomarkers for injury, and morphological changes associated with injury. The different segments of the nephron will be reviewed. Species and gender-related differences in renal structure and function will be emphasized, especially where these contribute to differences in nephrotoxic responses. These differences need to be considered when determining the relevance of animal studies to humans. We will review more commonly noted spontaneous lesions and their overall incidences, variance by strain (rodents) and age, all of which can impact study outcome. Lesions such as renal amyloidosis in the mouse and chronic progressive nephropathy in the rat are just two examples of spontaneous lesions which may adversely impact the outcome of a study or may be enhanced by chemical administration, chemical-induced findings and interpretation. Representative examples of segment-specific morphological changes that occur as a direct response to toxicant exposure will be provided, focusing on those changes evident in laboratory animals used for regulatory testing of new chemical entities. For each morphological change, a corresponding control will be provided to clearly depict the nature of the change. Finally, when choosing a biomarker to monitor for kidney effects, it is critical to understand the utility and limitations of traditional and novel serum and urinary markers of renal injury. Participants will gain a broader perspective on selection and implementation of biomarkers, particularly of the newer urinary markers which provide insight into segment specificity or mechanisms of nephrotoxic injury. Moreover, the participants will understand the specificity of each biomarker as a predictor of injury for specific parts of the nephron.

TECHNOLOGIES AND TOOLS FOR TOXICITY TESTING IN THE 21ST CENTURY.
R. Kavlock1 and D. Wilson2, 1U.S. EPA, Research Triangle Park, NC and 2Dow Chemical Company, Midland, MI.

Toxicology testing has traditionally been associated with defined and tiered testing around dedicated endpoints (i.e., acute, reproductive and developmental, chronic and cancer, etc.). Over time, validated surrogates or refined alternatives for some of the end-points have come into acceptance for screening and international regulatory use. Coinciding with the release of the NAS report on Toxicity Testing in the 21st Century: A Vision and a Strategy, a dedicated and rapid shift towards use of more non-whole animal testing is underway. Also, in vitro methods are expected to play a major role under REACH and to address the European Union-wide ban on animal use in cosmetics development. Inherent in this shift is a necessary understanding of the critical aspects of cellular, metabolic, and genetic functions affected in response to chemical and drug-induced toxicity as well as the dose-response attributes of the responses. Towards this end, elaboration of predictive toxicity pathways by integration of information from in vitro assays, surrogate organisms, ‘omics technologies, in silico approaches, and bioinformatics is ongoing. A review of how the classic approaches for toxicity testing are evolving into sophisticated molecular/mechanistic based approaches and the nature and implementation of in vitro high-throughput screening assays, with some mention of implementation of bioinformatics approaches will be addressed. Further insight into how the information will be considered in the context of animal use, testing prioritization, dose-response considerations, and human health risks will be explored. This basic course should be of interest to classically trained toxicologists and investigators and regulators wanting to understand the latest technologies and tools that will be the necessary repertoire for card-carrying mammalian toxicologists.

MECHANISTIC ROLE OF REACTIVE INTERMEDIATE PROTEIN COVALENT BINDING IN TARGET ORGAN TOXICITY: PAST, PRESENT AND FUTURE.
J. E. Masaouter1 and G. B. Corcoran2, 1Pharmaceutical Sciences, University of Connecticut, Storrs, CT and 2Pharmaceutical Sciences, Wayne State University, Detroit, MI.

The pioneering work of Brodie and co-workers in the early 1970’s demonstrated that protein covalent binding of a reactive metabolite of acetaminophen, N-acetyl-p-benzoquinonimine, was strongly associated with hepatotoxicity. Over the last three decades, immunological, biochemical, molecular biological, and proteomic approaches have been used to identify specific proteins adducted by reactive electrophilic metabolites. Although the identity of a number of protein targets, and the effects of covalent addition on protein structure and function are known, the precise role of protein covalent binding in chemical-induced toxicities remains a subject of contention. Indeed, the importance of reactive intermediate protein binding has been challenged by multiple studies employing experimental manipulations that reduce toxicity in the absence of an effect on protein binding. To adequately address these findings state-of-the knowledge of reactive intermediate protein binding and its toxicological consequences will be presented. The specific topics to be discussed include current views on the importance of protein covalent binding, latent in vivo and in vitro approaches to study covalent binding, the pharmaceutical industry’s perspective on the role of reactive intermediate binding in toxicity and the current safety assessment guidelines for drug candidates with covalent binding liability. Finally, current and future tools and technologies for studying reactive intermediate biology will be highlighted.

REACTIVE INTERMEDIATES AND THEIR INTERACTION WITH CELLULAR PROTEINS: HISTORICAL PERSPECTIVE.

Considerable advances in the understanding of mechanisms of formation of reactive metabolites and their molecular interaction with protein targets have been made since the initial pioneering work by Gillette, Mitchell and coworkers more than thirty years ago. However we still know very little about the mechanisms of subsequent toxicities as well as how to assess risks associated with the formation of reactive metabolites. Of particular interest primarily to pharmaceutical companies is the potential role of reactive metabolites in idiosyncratic adverse drug reactions. The presentation will provide a historical perspective on the role of reactive intermediate binding to cellular proteins and will also highlight the challenges in characterizing adducted proteins and their importance in toxicity.

THE ENIGMA OF REACTIVE METABOLITES.
J. Utrecht, Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.

Most drugs that cause idiosyncratic drug reactions (IDRs) form reactive metabolites, but not all drugs that form reactive metabolites are associated with IDRs. Two obvious factors are the fraction of the drug converted to a reactive metabolite and the total daily dose. Taking these factors into consideration during drug development would likely decrease but not eliminate IDRs. Not all covalent binding is associated with the same IDR potential and the question is what type is associated
with the highest risk. In order to answer this question it is essential to understand the mechanisms by which reactive metabolites can cause IDRs. It is likely that most are immune-mediated and two possibilities are that a reactive metabolite can act as a hapten or cause a danger signal. The fact that the same drug can cause a skin rash in one patient and liver toxicity in another suggests that there is a degree of random chance that determines whether T cells with the highest affinity are specific for a skin or liver drug-modified protein or even an unmodified protein leading to an autoimmune response. In fact, the same drug can cause two different types of toxicity in the same target organ, e.g. minocycline causes hypersensitivity liver toxicity in some patients with a relatively short time to onset while in others the liver toxicity is autoimmune in nature and occurs after a long period of treatment (>1 year). Therefore, the characteristics and presumably the mechanisms are different in different patients. It is likely that the location of reactive metabolite formation is a major determinant of the target of the IDR, but it is believed that an immune response in the liver requires immune activation outside of the liver. Therefore, reactive metabolite formation by macrophages may be important for the induction of an immune response. It has also been hypothesized that mitochondrial damage is important for induction of an immune response; therefore, reactive metabolite formation involving mitochondria may also be important. These hypotheses must be tested in order to understand which reactive metabolites are likely to cause IDRs.

Supported by grants from CIHR.

17 BIOACTIVATION AND COVALENT BINDING APPLIED IN A DRUG RESEARCH SETTING.

The discovery and development of new drugs has been faced with the very difficult problem of failure of new agents due to low-incidence severe human toxicity, such as hepatotoxicity, that is observed only during large phase 3 clinical trials or after the drug has reached the marketplace. Thus, there have been large efforts to develop methods that can be applied in the early stages of drug research to avoid developing compounds that could cause this. A focus of these efforts has been metabolic bioactivation of drugs to chemically reactive intermediates, a process long associated with various toxicities, such as carcinogenicity. Application of in vitro assays to assess the potential for bioactivation of new compounds in early drug research has become common. Such assays include nucleophile trapping assays and covalent binding assessments. However, their fidelity is not great enough to be able to distinguish those compounds that will have unacceptable levels of toxicity in humans from those that will be generally regarded as safe. Our findings have suggested that covalent binding data alone do not distinguish toxic from non-toxic drugs and that the use of this type of data in decision-making needs to be done with great care.

18 KNOWNS AND KNOWN UNKNOWNS IN PROTEIN COVALENT BINDING AND TOXICITY.
R. P. Handli, Department of Medicinal Chemistry, University of Kansas, Lawrence, KS.

The covalent binding (CBV) of xenobiotic metabolites to cellular proteins, first recognized in the 1940s, became firmly associated with acute cytotoxicity (and subsequent tissue/organ damage) in the 1970s. For many small molecules protein CBV correlates with cell injury, both in vivo and in isolated cells. Early studies emphasized adduct structure elucidation but target protein identification via C-14 labeling, 2D gels and mass spectrometry has recently accelerated. Unequivocal target identification requires the sequencing of adduct-bearing peptides but this is seldom achieved in in-life studies because the limits of detection are strained by proteins of low abundance, low fractional adduction and adduct heterogeneity. More commonly, when a single protein is identified in a radioactive spot it is assumed to be a covalently labeled target. Ironically, increased analytical sensitivity results in the identification of multiple proteins per spot, fewer peptides that can confidently be called targets. This problem can be reduced by reducing protease activity that contains an iso- topic signature, or that are amenable to pre-concentration by affinity capture via antibodies, streptavidin or click chemistry. Among >250 reported targets of >25 proteins, few are common to ≥ 4 proteins, and none appear to be an "Achilles heel" for the cell. Our overall knowledge of target proteins, and how they differ from non-target proteins, remains sparse. Global analysis of target lists has provided little insight into downstream mechanisms of toxicity, but bioinformatic pathway analysis of their interacting partner proteins reveals many to be involved in apoptosis, binding or response to unfolded proteins, and MAPK signaling. Future work should focus on 1) identifying more targets for more proteins, 2) targeted bioinformatic analysis leading to hypothesis testing, and 3) attempts to evaluate the cytotoxicity of adducted proteins directly (in the sense of Koch's postulates) by introducing them into cells without using small molecules or bioactivating enzymes. (Supported by NIH-GM-21784).

18A DRUG HYPERSENSITIVITY: MOLECULAR ASPECTS FROM MOLECULE TO MAN.

Adverse Drug Reactions (ADRs) are a significant cause of patient morbidity and mortality. Frustratingly from a therapeutic perspective ADRs in a small number of patients can lead to the withdrawal of drug that is safe and effective in the majority of patients, and thus prohibit effective drug therapy and may even lead to withdrawal of the drug. Such reactions can have variable toxicological intensity and variable frequency which may be increased in certain diseases. Our approach is to understand ADRs from molecule to man and back again in order to inform the physician, patient, medicinal chemist with respect to safe drug design. The present mechanistic understanding of drug hypersensitivity is firmly rooted in the hapten hypothesis which is based on the observation that penicillins become covalently bound to protein, thus forming a hapten which is recognized by various elements of the Immune system. Based on this premise it has been assumed that the generation of chemically reactive metabolites may initiate an immune response in a similar fashion. To accept this hypothesis, there are a number of critical unanswered questions which must be resolved. We need to know how a drug (or metabolite) can perform critical immunological functions (hapten, antigen, immunogen, co-stimulation) and what the basis of individual susceptibility is. To address these issues we have developed a panel of experts from the fields of inhalation, neurological, metal, and occupational toxicology will highlight neurological findings of animal and human studies after exposure to a variety of occupational particles and ambient air pollution. Cognitive deficits, brain abnormalities, and neurodevelopmental effects have been associated with exposure to metals in healthy children in Europe and North America. Our panel of experts from the fields of inhalation, neurological, metal, and occupational toxicology will highlight neurological findings of animal and human studies after occupational and environmental lung exposures. All aspects of the topic, such as metal chemistry, inhalation exposure of metal particles, metal translocation from the respiratory system to the central nervous system, and neurological responses, will be examined. An increase in the understanding of metal particle inhalation and neurotoxicity may allow for the development of prevention strategies to better protect susceptible populations in the workplace and environment.

19 NEUROLOGICAL RESPONSES AFTER EXPOSURE TO INHALED METAL PARTICLES.
J. M. Antonini1 and L. Chen2. 1NIOSH, Morgantown, WV and 2New York University, Tuxedo Park, NY.

Most studies examining the toxicology of inhaled metal particles have focused on responses in the target organ, the respiratory system. Less information exists regarding the effects associated with the inhalation of metals in extrapulmonary organs, specifically the central nervous system. There is increasing interest in the health effects of airborne incidental and manufactured metal nanoparticles (particles with one dimension <100 nm) in the environment and workplace. These smaller particles may translocate more easily from deposited sites in the respiratory tract to brain structures after inhalation. Mechanisms of particle translocation include uptake and transport along olfactory and sensory neurons, transcellular transport across respiratory epithelium to the circulation, and lymphatic clearance. Chemical composition, oxidation state, and solubility all may affect metal transport and biological responses to inhaled metals. Both animal and human studies have demonstrated that inhaled metals can translocate to the central nervous system, as well as, induce neurofunctional changes. Alterations in markers of neuroinflammation and cellular toxicity have been observed in specific brain regions using animal models after exposure to a variety of occupational particles and ambient air pollution. Cognitive deficits, brain abnormalities, and neurodevelopmental effects have been associated with exposure to metals in healthy children in Europe and North America. Our panel of experts from the fields of inhalation, neurological, metal, and occupational toxicology will highlight neurological findings of animal and human studies after occupational and environmental lung exposures. All aspects of the topic, such as metal chemistry, inhalation exposure of metal particles, metal translocation from the respiratory system to the central nervous system, and neurological responses, will be examined. An increase in the understanding of metal particle inhalation and neurotoxicity may allow for the development of prevention strategies to better protect susceptible populations in the workplace and environment.

20 OLFATORY TRANSPORT OF INHALED PARTICLES AND METALS.
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One route by which an inhaled particle can access the brain is direct retrograde axonal delivery via olfactory or trigeminal presynaptic nerve endings located in the nasal mucosa. This presentation reviews experimental approaches to evaluate olfactory transport, specific metals and ultrafine particles known to undergo olfactory transport, transport mechanisms, and the development of relevant computational
models. Experimental approaches and animal models used to verify that direct olfactory transport occurred have varied. Most studies examining direct "nose-to-brain" transport of a variety of materials have relied on intranasal instillation of the material with subsequent verification of the presence of the particle of interest in the olfactory bulb of animals. Other investigators have relied on the mechanical occlusion of one nostril prior to inhalation exposure. This procedure restricts olfactory transport to the side of the brain ipsilateral to the patent nostril. Other approaches have included direct visualization of translocated particles using analytical chemical imaging modalities and surgical transection of olfactory nerve fibers prior to particle administration. Certain metals (e.g., cadmium, manganese, iron, and thallium), drugs, and some solvents have been shown by these methods to undergo olfactory transport to the brain, where they may achieve tissue concentrations sufficient to initiate their toxic or pharmacologic effects. There is growing evidence that ultrafine particles can be taken up by the nerve endings and subsequently delivered to the brain via these retrograde transport processes.

A pharmacokinetic model describing the olfactory transport of manganese following acute inhalation exposures has been developed for the rat. The parameterized model was used to estimate the relative contribution of blood delivery versus olfactory transport in the rat. Several studies have also begun to explore the pharmacokinetic mechanisms involved in olfactory transport. For example, the organic anion transporters and divalent metal transporters likely play a role in the olfactory transport of manganese.

21 DOPAMINERGIC NEUROTOXICITY FOLLOWING EXPOSURE TO MANGANESE-CONTAINING WELDING FUMES.

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The potential for development of Parkinson’s disease (PD)-like neurological dysfunction following occupational exposure to welding fumes (WF) is an area of emerging concern. Welding generates a complex aerosol of fine and ultrafine metal particles that can potentially translocate from olfactory or pulmonary targets and accumulate in the brain. Manganese (Mn) in welding consumables is thought to be the causative factor for development of neurological deficits seen in welders. However, lack of definitive epidemiological evidence for such a causal association warrants further experimental investigation. To address this, Sprague-Dawley rats were exposed by whole-body inhalation or intratracheal instillation to a variety of WF that differ in elemental composition and solubility. Short-term inhalation exposure (40μg/m³; 3h/d x 5d) to Mn rich WF (GMA-SS; high chromium, less soluble) resulted in deposition of Mn in the olfactory bulb and striatum, altered the expression of divalent metal transporter 1 (Dmt1), dopamine D1 (Drd1) and D2 (Drd2) receptors, and induced a subtle neuroinflammatory response in the striatum and midbrain. On the other hand, a similar exposure to Mn rich steel (GMA-MS; low Mn, less soluble) in the same exposure conditions did not result in Mn deposition and no neurotoxic effects were observed. This study demonstrates the sensitivity of Mn rich WF to induce neurotoxic effects in rats.

22 CENTRAL NERVOUS SYSTEM EFFECTS AFTER EXPOSURES TO NANO-SIZED PARTICLES.

P Gillespie, G Kang and L Chen, New York University School of Medicine, New York, NY.

The respiratory tract is the primary target for inhaled nano-sized particles (NSP), but there is evidence of adverse effects in secondary organs like the brain. In general, the toxic potential of NSP has been attributed to two major characteristics: chemical composition and particle morphology with little attention given to the former. The aim of this study was to demonstrate the role of chemical composition in inducing neurotoxic responses. We exposed, by inhalation, C57BL/6J mice to either -50 μg/m³ of laboratory generated metals (Ni, Mn, and Cu) or non-metal (C, graphite) NSP of similar shape and sizes; or filtered air for 4h. Three brain regions (olfactory bulb, midbrain, and cerebellum) were harvested 1, 3, and 5 months after exposure. The three brain regions, a significant difference was only detected in the olfactory bulb (n=8/group, P<0.05) followed by detectable amounts in the midbrain even after cessation of exposure (100μg/m³ for 5h/d, 5d/w, for up to 5m). The three brain regions were collected 1w, 3m, and 5m, 24h after the last day of exposure for gene expression analyses (n=4/group). PCR profiling systems for OS and inflammatory pathways were used to evaluate change in expression of 168 genes (confirmed by individual RT-PCR for genes with 2x or high change). Collectively the following rank for altered gene expression was observed: olfactory bulb > midbrain >> cerebellum. In summary, acute and chronic studies suggest that all three regions are affected by inhaled Ni NPs via oxidative stress-induced inflammatory mediated pathways; but, the olfactory bulb may be a more sensitive target to adverse effects.

23 NEUROBEHAVIORAL EFFECTS IN ADOLESCENTS EXPOSED TO METALS.

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Background: Increased parkinsonism was observed in Valsamonica, a valley in the Italian Alps. Prevalence was higher in the vicinity of ferroalloy plants and associated to the manganese level in deposited dust. The aim of this study was to assess motor and cognitive functions in adolescents in the exposed area. Methods: We retrospectively examined in PM10 air particles collected in industrial area and rural sampling. Samples were analyzed with Total Reflection X-Ray Fluorescence. Soil was analyzed at surface and 10cm depth. Adolescents of 11-13 years old were recruited through the local school system for neurobehavioral examination. Various biomarkers were collected for metal analysis. Results: A total of 303 children residing in the exposed area and a reference area participated in this study. Average airborne manganese was 57.79 ng/m³ (n=86, range 1.24-516.70) in Valsamonica and 22.45 ng/m³ (n=11, range 5.30-36.59) in the reference area. Lead, iron, zinc and chromium also showed significantly higher levels. Manganese results were significantly higher also at the surface and at 10 cm depth of soil and in salad. Children in the exposed area showed increased levels of motor coordination and odour identification associated with airborne manganese at multivariate analysis. Blood lead was inversely associated with IQ, but only in the metal exposed area of Valsamonica. Conclusion: Environmental exposure to manganese in adolescents is related to deficit in motor and olfactory functions whereas concomitant lead exposure is related to decrease of IQ. Acknowledgement: This work was partially supported by the EU through its Sixth Framework Programme for RTD (contract no FOOD-CT-2006-016253). It reflects only the authors’ views. The European Community is not liable for any use that may be made of the information contained therein.

24 NEUROINFLAMMATION, SEVERE AIR POLLUTION AND CHILDREN.

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Exposure to air pollution is associated with systemic inflammation in healthy children in Mexico City (MC). Children are at risk since childhood and adolescence are crucial periods of brain development associated with dynamic behavioral, cognitive and emotional changes. Children living in MC exhibit evidence of chronic inflammation of the upper and lower respiratory tracts, alterations in circulating inflammation mediators, breakdown of the nasal respiratory epithelial barrier and the blood-brain barrier (BBB), ultrafine particle matter (UFPM) in frontal endothelial cells, and elevated levels of plasma endothelin-1. Fifty-six % of MC City children show prefrontal white matter hypertensive lesions by MRI compared to controls in low pollution areas. Autopsy studies measuring mRNA COX2, IL-1β, and CD14 in target brain regions from low or highly exposed residents 25.1±1.5y, have shown upregulation of COX2, IL-1β, and CD14 in olfactory bulb, frontal cortex, substantia nigra and vagus nerves, disruption of the BBB, endothelial activation, oxidative stress, and inflammatory cell trafficking in highly exposed subjects. Amyloid beta 42 immunoreactivity was observed in 58.8% of APOE 3/3 <23y, and 100% of the APOE 4 subjects. Brainstem accumulation of amyloid beta 42 and alpha synuclein and microgliosis are present in exposed children in keeping with the infra-tentorial inflammation. Alterations in measures of fluid intelligence and cognitive control along with alterations in auditory brainstem pathways predict school performance, complex learning, reasoning and the ability to block impulsive anti-social
The ovary is responsible for the differentiation and release of a mature oocyte for fertilization and for synthesizing and secreting hormones that are essential for follicle development, estrous cyclicity, and maintenance of the reproductive tract and its function. Reproductive toxicity studies are important components of the regulatory approval of drugs and chemicals. The identification of ovarian toxicity and determination of its cause requires familiarity with ovarian anatomy, physiology, relationships with other components of the female reproductive tract, and the neuroendocrine regulation of the estrous cycle. A mechanistic approach at the morphologic, biochemical, and molecular level demonstrate that various factors are involved in ovarian toxicity. Therefore, our focus will be on the basic concepts of ovarian anatomy, histopathology, and potential mechanisms of toxicity. We will begin by discussing the importance of assessing fertility that utilizes a combination of methods including evaluation of estrous cycle length, fertility endpoints, and ovarian weights. Recent collaborative work suggests a 2-week rodent study may be sufficient to elucidate the effect of pharmaceuticals on ovarian function and its impact on the revised ICH M3 will be presented. Better interpretation of drug-induced ovarian toxicity will be highlighted as fertility effects in rodents, especially when both sexes are treated do not often distinguish between male or female mediated effects. A mechanistic model of ovarian toxicity of 4-vinylcyclohexene diepoxide provides an understanding of the potential risk of human exposure to environmental ovarian toxicants and greater insight of toxicants on reproductive health in women will also be discussed.

The ovary provides a favorable environment for the production and maturation of gametes in the female. Ovarian toxicity is of concern primarily because of potential impacts upon a female's reproductive capacity. Assessments of reproductive endpoints in specially designed studies are, perhaps, the most sensitive means of detecting ovarian toxicity. However, in the chemical and pharmaceutical industries, these tests are usually not conducted until late in compound development. For this reason, histological examination of tissues in preclinical safety studies of xenobiotics may provide early evidence of compound-related ovarian toxicity. To this end, pathologists and toxicologists must be familiar with normal ovarian anatomy and physiology, cyclical relationships with other components of the female reproductive tract, and endocrinology of the ovarian cycle. Using the rodent as an animal model, this presentation will provide a brief review of the anatomy and histology of the ovary and other organs of the female reproductive tract, their variation during the ovarian cycle, and the neuroendocrine bases for these changes and (2) a discussion of mechanisms by which ovarian toxicity may occur, some of the resulting morphologic changes, and potential issues related to the interpretation of results.

For chlorofluorocarbons and 1, 1, 1-trichloroethene causes ovarian dysfunction by damaging primordial follicles and their oocytes in female rats. Anticancer drugs and radiation therapy are the most common of the known ovarian toxicants. The cytotoxic anticancer drugs affect dividing cells, and are expected to affect the granulosa and theca cells of the ovary. In addition, alkylating drugs such as cyclophosphamide are the most potent at inducing ovarian failure. Prolonged amenorrhea due to premature ovarian failure leading to infertility is one of the serious side-effects of cyclophosphamide. The susceptibility of the ovaries to different compounds depends on the stage of ovarian development at which exposure occurs and the consequences may be irreversible if exposure occurs at a critical stage in ovarian or hypothalamic differentiation. In addition sites and possible known mechanisms of actions of ovarian toxicants will be discussed in details.
SILICA AND ASBESTOS IMMUNOTOXICITY: MECHANISMS TO FIBROSIS, AUTOIMMUNITY, AND MODIFIED TUMOR RESISTANCE.

A. Holian, University of Montana, Missoula, MT.

Effects of silica/asbestos on local and systemic immune system components are very important in the cascade of events in a host that evolve over the course of time from the point of initial exposure to the ultimate onset of lung fibrosis (i.e., silicosis, asbestososis), malignant tumors (i.e., lung cancer, mesothelioma), or autoimmune disorders (e.g., systemic sclerosis, rheumatoid arthritis—Caplan syndrome). In particular, mechanisms used by immune competent cells to process the entrained silica or asbestos may affect induction of these pathologies. With regard to asbestos specifically, there may also be a reduction in local/regional anti-tumor immune responses that serves to amplify its own carcinogenic potential in situ. We will begin with an up-to-date overview of emerging topics in the field of silica/asbestos toxicology that can, in turn, serve as a basis to understand mechanistic interpretations that link development of pneumoconioses to fibrotic diseases, autoimmunity, and cancer. To better understand these issues the latest findings on the roles that particle recognition, inflammation, apoptosis, cytokine-driven inflammation, or immune dysfunction have in eventual induction of fibrosis, altered autoimmunity, and/or modified tumor resistance in silica/asbestos-exposed hosts. It is anticipated that with an enhanced understanding of the molecular pathological mechanisms underlying the immunotoxicologic effects of silica/asbestos, researchers in many fields (including immunology, immunotoxicology, pulmonary biology and medicine, occupational medicine) will be better able to develop therapeutic tools for the prevention, mitigation, or treatment of debilitating diseases induced by these agents.

SCAVENGER RECEPTORS AND MACROPHAGE SUBPOPULATIONS IN THE DEVELOPMENT OF SILICOSIS.

A. Holian, S. Thakur, C. Beamer, C. Migliaccio and R. F. Hamilton, University of Montana, Missoula, MT.

Macrophages are a key cell in the initiation and propagation of lung inflammation resulting from exposure to fibrogenic dusts such as crystalline silica. Recent data supports a critical role for activation of the NLRP3 inflammasome, with release of IL-1β family of cytokines, in the inflammatory process. Class A scavenger receptors (MARCO and to a lesser extent SRA/II (CD204)) are responsible for the majority of silica binding and internalization by alveolar macrophages from different strains of mice. In addition, MARCO mediated silica uptake has been shown to be responsible for initiating macrophage apoptosis. Unexpectedly, the absence of MARCO decreased silica-induced apoptosis, but increased IL-1β release in vitro and increased acute and chronic inflammation in vivo suggesting different pathways are involved in silica uptake. Therefore, MARCO-mediated uptake appears to increase the activity of apoptotic caspases while non MARCO-mediated uptake increases caspase-1 activity through the NLRP3 inflammasome following lysosomal destabilization and release of cathepsin B. The non MARCO-mediated pathway of silica uptake (and subsequent NLRP3 inflammasome activation) can be blocked by depletion membrane cholesterol with methyl-β-cyclodextrin or by inhibiting Syk kinase with picatetanol, suggesting an important role for lipid organized regions of the macrophage membrane in the mechanism of silica uptake. Consequently, scavenger receptors on macrophages are important in controlling the silica-induced inflammatory response. Therefore, variation of scavenger receptors on macrophage subsets in humans may help explain the known variation in human susceptibility to developing silicosis and contribute to silica-induced autoimmune disease development. The work was supported in part by NIH grant ES-015294.

ASBESTOS-INDUCED AUTOIMMUNITY: THE POSSIBLE ROLE OF SYSTEM XC- IN MACROPHAGE SIGNALING.

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Inhalation of asbestos, as well as silica, is associated with the production of autoantibodies, and an increased risk of systemic autoimmune disease as well as fibrosis and cancer. Because there is a serious need for treatment options in both autoimmune and fibrotic diseases, a better understanding of the mechanisms leading to the loss of tolerance to self antigens is essential. Exploration at the cellular level reveals that antigen presenting cells (APCs) may be at the heart of lost immune tolerance through subtle changes in critical redox signaling. System xc- is an amino acid antiporter that exchanges intracellular glutamate for extracellular cysteine, which is subsequently reduced to cysteine, the rate-limiting precursor for glutathione (GSH) synthesis. The function of system xc- mediates two essential redox relevant processes – it maintains intracellular GSH as a protective antioxidant and maintains levels of extracellular glutamate shown to be critical for activating signaling pathways in adjacent cells. Interestingly, we have found evidence that both of these processes may be involved in the response of APCs to asbestos exposure. Using RAW 264.7 macrophages, we have found that asbestos, but not silica, significantly upregulates expression and activity of this transporter. System xc- activity appears to be protective since pretreatment with glutamate, a substrate inhibitor, led to cell death in the presence of asbestos, but both N-acetylcysteine and cystine significantly protected macrophages from asbestos toxicity. Further, B cells cultured in the
effect approach to evaluate non-carcinogenic risk. However, ATB is not specific to cancer, but can apply to any adverse health effect. ATB per se does not provide a value for the low dose slope, although it has been argued that linearity extrapolation from high doses is unlikely to provide an underestimate. Recent reports from a National Academy of Science committee (NRC 2008) and from a workshop organized by John Hopkins and EPA (White et al. 2009) both used ATB to support recommendations for using linear models for low-dose risk assessment except under specified stringent conditions. This talk will discuss issues that need addressing when assessing the ATB argument. One difficulty in putting out linearity as predicted by ATB is that any augmentation of an ongoing process – no matter how slight – is expected to lead to low dose linearity. Alternative theories of low-dose response predict safe threshold doses or U-shaped curves derived from homeostatic and defense mechanisms, but generally do not address ATB directly. Data, no matter how detailed, are never able definitively to distinguish between a true no-effect dose and a linear response because the statistical uncertainties will be large enough to admit both. References: Crump KS, Hoel DG, Langley C, Peto R. (1976) Cancer Research 36:2973-2979. NRC. (2008) Science and Decisions: Advancing Risk Assessment. National Academies Press. White RH, L. Zeise L, Fox M, Dominici F, Burke TA, White PD, Harris DB, Samet JM. (2009) Environmental Health Perspectives 117(2):283-287.

The recent report by the National Research Council “Science and Decisions: Advancing Risk Assessment” advocates for a unified approach to dose response assessment for carcinogens and non-carcinogens. The report lays out three conceptual models of dose response assessment. For the first conceptual model, for any particular person the dose response is non-linear, that is threshold-like, but because of differential sensitivity within the population, ongoing disease processes and high background exposures, the dose response relationship at the population level is linear at low doses. For the second conceptual model, low-dose linearity can be rejected based on considerations of background, vulnerability and heterogeneity: The dose response is threshold-like for both individuals and the population. For the third conceptual model, low-dose linearity holds for the individual as well as the population. The report advocates a formal systematic approach for selecting among conceptual models, involving the assessment of possible background disease processes and exposures, vulnerable populations, and consideration of potential modes of action. In our presentation we explore model selection for non-carcinogens and carcinogens that have primarily non-genotoxic modes of action.

Although the existence of thresholds has been hypothesized for most types of toxicity, it has (with one or two exceptions) not been possible to demonstrate those thresholds empirically using traditional toxicity methods. The statistical power to detect small increases in incidence requires too many subjects to make such experiments practical. The advent of technologies such as genome-wide gene expression analysis, along with increasing knowledge about mechanisms of toxicity, makes it possible to identify the key molecular cellular events that underlie toxicity and for which small changes can be measured reliably. One example of how these two important streams can be coupled is the observation of dose-dependent transitions in the expression of genes in inflammatory pathways in the nasal epithelium of formaldehyde-exposed rats (Andersen et al. 2008, Tox. Sci. 105:368-83). There was no evidence of inflammation and nasal scores were essentially the same in the control group, so it appears the background is insufficiently high for low-level formaldehyde exposure to drive a response. An example from my group is the investigation of dose-response for gene expression in the fetal or juvenile rodent reproductive system after administration of estrogenic compounds. Pregnant rats were exposed three estrogens at dose levels spanning 5-6 orders of magnitude. Gene expression was evaluated in the fetal testes. The dose-response curves for gene expression were monotonic, with no changes in gene expression at the lowest dose levels. There is an existing estrogen load in the fetus, although it is tightly regulated. The results suggest that there is insufficient bioavailable endogenous estrogen to act as a background to which exogenous estrogens are additive. There are other examples for which enough is known about the mechanism of toxicity that we can determine the key events that should be monitored, such that empirical evaluation of whether the background of existing disease is additive and sufficient enough to negate the presence of a threshold, additive but insufficient, or independent.
It has been argued that if an agent enhances the underlying biological processes producing the background rate of disease in a population, then small increments of dose will have a nearly linear effect on increasing the rate of disease. To evaluate this proposition and its importance in practice for noncancer risk assessment, a clear idea is needed about the necessary assumptions regarding the nature of background disease, its dependence on values of (and underlying variation in) physiological variables, and the means by which small increments of dose are able to change the distribution of those key physiological variables. The dependence of dose-response on continuous variation in causal and “susceptibility” factors versus its dependence on stochastic events that change qualitative states in disease progression needs to be clear. I argue that the needed assumptions to achieve low-dose impact are more restrictive than is generally recognized, and that they may not jibe with the usual understanding about the properties and nature of both noncancer toxicity processes and background disease incidence. As we increasingly evaluate noncancer toxicity modes of action, and as we develop a systems-biology approach to understanding them, it will be important to bring these principles to bear in evaluating the potential for effects at low doses in heterogeneous human populations.
strongly focused on delayed ventricular repolarization (QT prolongation), some-
what to the exclusion of other indices of CV system function and structural effects. A consensus from a June 2009 HESI Structural and Functional CV Risk Assessment Workshop was that drug effects on peripheral hemodynamics and car-
diac function (blood pressure and inotropic state, respectively) were as important as
electrophysiologic effects to the clinical safety of drugs. The group agreed that an
tegrated evaluation of structural and functional CV drug effects is important in
understanding drug safety and determining risk to human patients as functional ef-
fects may lead to structural damage and, conversely, structural damage may pro-
duce undesirable functional effects. The group also agreed that previously em-
ployed nonclinical methods to evaluate functional end points were of uncertain
value due to variable scientific rigor and/or poorly characterized methodology
sometimes used in safety pharmacology and toxicology studies. Specifically, work-
shop participants noted the limited chronic evaluation of functional endpoints
(electrophysiologic and nonelectrophysiologic) achieved in repeat dose nonclinical
studies. This issue has achieve prominence in recent years due to the increasing
presence of large molecule drug therapies for which acute, crossover safety pharma-
cology studies may not be appropriate. This presentation will describe the current
state of nonclinical CV safety assessment, identify gaps, and describe ongoing activ-
ities of a HESI working group on CV risk assessment to develop improved non-
clinical predictive strategies for the human CV safety of small and large molecule
drug candidates.

W 46 THE CARDIAC SAFETY RESEARCH CONSORTIUM (CSRC): A CRITICAL PATH INITIATIVE FOR CARDIAC SAFETY EVALUATION THROUGH A NEW PARADIGM OF PRE-COMPETITIVE PUBLIC-PRIVATE PARTNERING.

M. W. Krucoff. Duke University Medical Center, Duke University, Durham, NC. Sponsor: S. Petti.

The escalating costs of research and development has been directly associated with a
falling number of successful new molecular entity filings in the new millennium, providing
the impetus for the launch of the Critical Path Programs by FDA in 2004. Cardiac safety issues provide one of the most significant, complex and risk-
laden aspects of new compound assessments, from early pre-clinical signals through
all phases of pre-and post-market human exposure. Through a memo of under-
standing between Duke University and the U.S. FDA, the CSRC provides a trans-
parent public-private partnering structure to cultivate, develop, launch and imple-
ment pre-competitive programs targeting key issues in cardiac safety evaluation.
Fundamental to the CSRC is the emphasis on pan-stakeholder collaboration, which in itself provides access to a unique range of industry, regulatory and aca-
demic/clinical skill sets focused on developing better, less burdensome cardiac safety evaluation strategies and processes. In this presentation, the structure and function of the CSRC will be reviewed, as well as a series of brief overviews of CSRC deliverables and active programs to date. Such programs include the CSRC ECG-QT Public Domain Data Warehouse, thinktank/incubator programs that
have launched unique public health initiatives in obligatory drug-device safety in-
teractions (such as dual anti-platelet therapy and drug eluting stents, and atrial fib-
rillation ablation safety national registry), and a new generation of peer review liter-
ature generating consensus white papers in focused areas of interest (such as
evaluation of oncology compounds and the translation gap between pre-clinical
and clinical cardiac safety testing).

W 47 THE PRESENT AND FUTURE OF STEM CELLS IN DRUG DEVELOPMENT.

K. L. Kolaja1, C. K. Parker2, R. McKernan3, D. Fink1 and J. Thomson4, 1Nonclinical Safety, Hoffmann-LaRoche, Nutley, NJ, 2Cellular Dynamics, Madison, WI, 3CBER, Food and Drug Administration, Gaithersburg, MD, 4Regenerative Medicine, Pfizer, Groton, CT and 5Regenerative Biology at the Morgridge Institute for Research and School of Medicine and Public Health, University of Wisconsin, Madison, WI.

Pluripotent stem cells have the potential to differentiate into any cell type in the
body. This biological paradigm is being leveraged to change the way drugs are dis-
covered, assessed, designed, and delivered. Stem cell derived models of various tis-
sues including inflammatory cells, cardiomyocytes, neurons, beta-oid cells, etc.,
have demonstrated utility in understanding disease processes as well as predicting toxico logical outcomes. As our understanding of genetic reprogramming into a
stem cell and subsequent differentiation into terminal cell types increases, it will en-
able a variety of applications in the pharmaceutical and chemical industries. In vitro
models will be followed by individual understanding of biology through inducible pluripotent (iPS) technology and ultimately cellular therapies will be brought to the
clinic. In this session, we will cover a series of presentations that will expand upon
our understanding of pluripotent cell types and their utility in providing cellular models,
how they are being used to understand pharmacological mechanisms of efficacy and
make regenerative, cellular therapy a reality.

W 48 HUMAN INDUCIBLE PLURIPOTENT STEM CELL DERIVED IN VITRO MODELS - THE PATH TO A BETTER UNDERSTANDING OF INDIVIDUAL BIOLOGY AND THEIR UTILITY IN DRUG DISCOVERY AND DEVELOPMENT.

C. Parker. Cellular Dynamics, Madison, WI.

iPS cells (induced Pluripotent Stem cells) have the ability to act as a starting mate-
rial to produce any cell type from any genetic background in the body. Providing in
vitro models to understand pharmacological mechanisms in a human model that
can represent the diversity in human biology will provide a more predictive model
to understand toxicity through the production of normal cells. Human iPS derived
Cardiomyocytes is an example of an in vitro model that will provide better mecha-
nistic understanding of a variety of compounds and how they affect individuals dif-
frently. This presentation will provide data highlighting the genetic models that
have been produced that demonstrate unique cardiotoxic mechanism and the util-
ity in predictive toxicology. Additionally, the ability to generate additional stem cell
derived cell types as well as the relevance of various adult tissues as starting materi-
als for stem cell creation will also be discussed.

W 49 APPLICATION OF STEM CELL- DERIVED CARDIOMYOCYTES IN TOXICOLOGY AND SAFETY PHARMACOLOGY.

K. L. Kolaja. Nonclinical Safety, Hoffmann-LaRoche, Nutley, NJ.

Stem cell derived in vitro models are a potential means to improve the prediction of
human toxicity early in the safety assessment process. Cardiomyocytes are one of
the first cell types to be reproducibly and consistently produced in scale which af-
fords an opportunity for toxicologists given the relative frequency in which mole-
cules are terminated from development due to cardiovascular toxicity. To test this
model, stem cell derived cardiomyocytes were assessed across a diverse set of known
cardiotoxic molecules for their mechanistic effects on proliferation, cell death, ki-
ase signaling cascade, and mitochondrial function. Examination of the results in-
dicate that human cardiotoxicity can be accurately identified using stem cell de-
derived cardiomyocytes. In addition, electrophysiological experiments using patch-
clamping and multi-electrode arrays have been conducted to confirm the expres-
sion and function of known cardiac conduction channels. Similarly, stem cell de-
derived cardiomyocytes are able to beat in culture using detectable channel activity
that is responsive to drug treatment. Our results reveal the stem cell derived car-
diomyocytes are able to effectively identify molecules that are cardiotoxic.

W 50 PHARMACEUTICAL PERSPECTIVES ON INTRODUCTION OF REGENERATIVE MEDICINE CONCEPTS INTO THE EXISTING PHARMACEUTICAL PARADIGM.

R. McKernan and J. D. McNeish. Regenerative Medicine, Pfizer, Groton, CT.

This presentation will provide the perspective of cell based therapies from a phar-
aceutical company perspective. Cell based therapies, both autologous and allo-
genetic, have demonstrated clinical utility yet the intersection of such therapies
within a pharmaceutical company raises novel questions including definition and
characterization of the product, clinical trial design, dose selection, indication as-
essment, etc. Establishing a clearly defined strategy that addresses the above while
taking into consideration medical need and patient safety and clinical improvement
over existing therapies will be the challenge as our understanding of stem cells in
general improves. The challenge is to translate basic early biology into tangible
commercial opportunities within the existing pharmaceutical paradigm. This talk
will provide a deeper perspective on how these issues may be addressed.

W 51 DEVELOPING STEM CELL-BASED THERAPIES: CBER PRECLINICAL REGULATORY CONSIDERATIONS.

M. Serebian. CBER, Food and Drug Administration, Gaithersburg, MD. Sponsor: K. Kolaja.

The steady accumulation of scientific information related to the biological prop-
erties of stem cells supports their potential to provide novel cellular therapy and re-
generative medicine products for treating a broad spectrum of diseases. Stem cell-
Cell-based therapies, similar to many previously over-hyped therapeutic methods, run the risk of failure due to premature introduction to patients. This presentation will discuss issues and concepts related to inducible pluripotent and embryonic stem cell technologies and the barriers and challenges that will need to be overcome to before these technologies become a viable therapeutic approach. Issues to be considered will consist of establishing a better understanding of what constitutes an inducible pluripotent stem cell as it relates to their characterization for pluripotency and safety. Our basic biological understanding of what comprises a “stem cell” is still at a fairly descriptive level based on self renewal and their ability to differentiate into different germ layers. Further understanding of these characteristics will be required to ensure their safety in a clinical setting. Establishing tools to efficiently understand multiple characteristics of pluripotency as new methods of induction of pluripotency develop will be essential to establish a use case and baseline to further address their utility.

**PL 52 HOW THE UNDERSTANDING OF THE BIOLOGY OF INDUCIBLE PLURIPOTENT STEM CELLS WILL EVOLVE: PREDICTIONS ON METHODS OF STEM CELL INDUCTION AND THE IMPACT ON CELL-BASED THERAPY.**


**Pl 53 MICRNORNAS AS POTENTIAL CIRCULATING BIOMARKERS FOR TESTICULAR TOXICITY SCREENING.**

H. Lin, J. Milano, M. Muhkamedova and D. Yousse. Safety Assessment, AstaZeneca Pharmaceuticals, Wilmington, DE.

Testicular toxicity is an occasional finding for drug candidates in preclinical development. MicroRNAs (miRNAs), a family of small non-coding RNAs involved in regulation of normal gonadogenesis and spermatogenesis, have emerged as novel biomarkers and potential therapeutic targets. To determine whether circulating miRNAs might be useful as non-invasive biomarkers for testicular toxicity, we performed expression profiling of known testicular expressed miRNAs in adult rats with a SYBR green-based quantitative PCR method. We found that 10 miRNAs are detected at substantial levels in the blood but with distinct expression patterns from the tests. Target genes of miRNAs we’ve predicted by miRBase targets prediction tools show a correlation with steroidogenesis and spermatogenesis. Pathway analysis revealed that the predicted target genes are concentrated in select biological pathways, exemplified by reproduction GnRH signaling pathway, cell cycle regulation, and cell adhesion/junctions regulation. In further analysis miRNA alterations associated with the toxicity of testicular toxican were investigated using *in vitro* models. We found that miRNA expression is dysregulated with altered miRNA profiles in rat Sertoli SerW3 cells treated with testicular toxicant 2,5-Hexaamine. In a search of targets of miRNAs, miR24 was predicted to target Cyp11a1 (p450sc), the first and rate-limiting step enzyme in steroidogenesis. Stimulation with gonadotropin hCG or potent cAMP analog 8-bromo-cAMP in rat Leydig LC540 cells led to a significant decreased miR24 expression and increased Cyp11a1 mRNA expression. In conclusion, our results indicate the potential role of miRNAs in regulation of testicular functions. Thus, circulating miRNAs may serve as non-invasive biomarkers of testicular function.

**PL 54 EARLY DETECTION BIOMARKERS OF GASTROINTESTINAL TOXICITY IN RATS.**


Gastrointestinal (GI) injury is a common side effect of therapeutic interventions, especially in anti-proliferative cancer drugs. Although several biomarkers for GI toxicity exist, they appear only after significant organ damage has occurred. The ob-jective of this study was to evaluate and identify potential sensitive biomarkers of GI toxicity from blood and feces to screen compounds. Male rats were dosed orally for five days with vehicle, 5 mg/kg and 15 mg/kg of a known PAK4 inhibitor developed for anti-cancer therapy which induces GI toxicity. Blood and fecal samples were collected pre-dose and at various timepoints, and analyzed for potential GI toxicity biomarker levels. At termination, the GI tract was collected for histologic and morphologic evaluation. Clinically, GI injury appeared on day 4 in the 15 mg/kg group and included soft and watery feces, decreased activity and skin turgor, and hunched posture. In the plasma, L-citrulline levels showed a significant dose and time dependent decreases compared to controls, whereas diamine oxidase levels showed no meaningful biological correlation. In the feces, daily bile acid levels gradually increased with a peak on day 4; calprotectin levels were detectable but low at all time points. Fecal levels of the GI-enriched miRNA miR-194 showed treated group elevation on day 3 in both treatment groups. Numerous erosions and ulcerations were detected by nitrotetrazolium staining of the GI tract in the 15 mg/kg treatment group. Additionally, adverse histologic findings were seen only in the 15 mg/kg/treatment group which consisted of epithelial necrosis, chronic active inflammation, erosions/ulcers, and edema in the GI tract. In summary, blood L-citrulline appears to be a sensitive and predictive biomarker for early detection of GI toxicity in the current rat model. In addition, specific endogenous miRNA species in the feces could serve as a potential novel and informative biomarker for GI injury as demonstrated in the study.

**PL 55 DEVELOPMENT OF EXHALED BIOMARKERS FOR AIRWAY DISEASE AND EXPOSURE.**

K. Bloemen1, R. Van Den Heuvel1, E. Gorats1, G. Koppen1, E. Witters1, K. Desager3 and G. Schoeters1, 4. Environmental Risk and Health, VITO, Mol, Belgium, 2Center for Proteome Analysis and Mass Spectrometry, University of Antwerp, Antwerp, Belgium, 3Department of Pediatrics, University Hospital Antwerp, Antwerp, Belgium and 4Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium. Sponsor: B. De Wever.

Exhaled breath condensate (EBC) contains trace amounts of secreted pulmonary proteins, and may be useful in the search for biomarkers for airway disease and environmental exposure. We tested the hypothesis that EBC contains non-invasive markers that are related either to wheezing, allergy, or the asthma predictive index (API) and/or to outdoor exposure parameters. EBC was collected and exhaled nitric oxide (eNO) was measured in 3-year-old children in a follow-up of the Flanders Environmental and Health Study (FLEHS). EBC pH was measured 5min after collection without deaeration. We developed a protocol to analyze the proteins in EBC*. In short, proteins in 1 ml EBC (about 1μg) were concentrated on beads and digested with trypsin. Resulting peptides were separated and analyzed by nanoLC/MSMS. The pattern was selected by performing Support Vector Machine analysis. Stepwise multiple regression analysis was used for data analysis. eNO was significantly correlated with wheezing and allergy, but not with API. EBC pH was not correlated with any of these clinical outcome parameters, but was influenced by outdoor NO2 and O3 concentrations 8 days prior to examination. An EBC protein pattern based on 8 peptides was able to classify API positive and negative children correctly. Although the nature of these proteins remains to be identified, the approach provides an important opportunity for the development of non-invasive biomarkers for early asthma diagnosis and for linking environmental exposure with effect biomarkers. *Proteomics Clinical Applications 2009: 3(4): 498-504. This study was supported by the Belgian Science Policy (Contract number SD/HE/05A) and by the Flemish Government (Development, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy.

**PL 56 PROFILING OF MICRNORNA EXPRESSION IN THE LIVERS OF RATS ADMINISTERED WITH CARCINOGENIC DOSE OF COMFREY.**

Z. Li and T. Chen. Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR.

MicroRNAs (miRNAs) are small non-coding RNAs that function as regulators of gene expression to control cell growth and differentiation. MicroRNAs are substantially altered in various types of tumors and have been used as biomarkers in defining malignant status. However, studies on responses of miRNA expression to carcinogen insults in their target tissues are rare. In this study, we analyzed miRNA expression in the livers of rats treated with carcinogenic dose of comfrey (Symphytum officinale), a rat botanical carcinogen, for 12 weeks. Groups of 6 Big Blue Fisher 344 rats were fed a normal diet or a diet containing 8% comfrey root. The animals were sacrificed one day after the last treatment and the livers were isolated from the control and treated rats for miRNA expression analysis. The miRNA expressions were determined using miRNA microarrays for human, mouse and rat
the ALT inhibitor. This study demonstrates the utility of MDH, PNP and GLDH findings regardless of whether the APAP-administered rats were also treated with ity effectively limiting the utility of ALT as a biomarker for hepatotoxicity. In con-
to naïve and APAP treated animals caused a dose dependent decrease of ALT activ-
ence. Therefore, the development of alternative biomarker approaches is imperfect. These discrepancies could be due to adaptive responses, altered liver correlation between increased ALT levels and morphological liver findings is rather
Hepatotoxicity provides a major challenge in drug discovery. While alanine amino-
to bile acids are important in many metabolic processes and toxic events. Their role in hepatotoxicity was investigated by targeted metabolomics analyses within the EU FP6 InnoMed/PredTox consortium, a proj-
ct with 20 partners from pharmaceutical industry, SMEs and universities. Thirty non-, glycine- and taurine-conjugated bile acids were analyzed by LC-MS in serum and urine of rats from 14 day studies collected on days 1, 3 and 14. Bile acids were affected differently depending on the type of liver damage. Compound 4BA, which damaged the bile duct epithelium, induced a strong increase (up to 8000 fold) of mainly the glycine- and taurine-conjugated bile acids in serum and urine e.g. gly-
2T oxicologic Pathology, Pfizer Inc., Groton, CT.

Bile acids as potential biomarkers for different types of liver damage – a targeted metabolomics LC-MS approach.

57
A. Amberg1, F. Durrieu1, M. Sieber2, M. Raschke3, B. Riefke3 and H. Ellinger3.

Bile acids are potential biomarkers for different types of liver damage – a targeted metabolomics LC-MS approach.

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A. Amberg1, F. Durrieu1, M. Sieber2, M. Raschke3, B. Riefke3 and H. Ellinger3.

Besides their lipid solubilizing property, bile acids are important in many metabolic processes and toxic events. Their role in hepatotoxicity was investigated by targeted metabolomics analyses within the EU FP6 InnoMed/PredTox consortium, a proj-
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nase regulates Hsp27-induced reorganization of the actin cytoskeleton in autoim-
mune blistering diseases. p38 MAP kinase has also been reported to regulate ex-
pression of caveolin-1, the major component of caveolae, a membrane structure im-
portant in regulating signaling in epithelial cells. In the present studies we charac-
terized changes in the p38 MAP kinase signaling pathway using an in vitro skin 
model in which mouse keratinocytes are grown at an air-liquid interface and 
exposed directly to CEEs, a model sulfur mustard vesicant. CEEs treatment (100-
1000μM) caused a concentration-dependent increase in actin polymerization in 
keratinocytes labeled with rhodamine-phalloidin and analyzed by confocal microscopy. 
CEE also upregulated Hsp27 and caveolin-1 expression and activated the 
p38 MAP kinase signaling pathway through increased phosphorylation of p38 
MAP kinase. Treatment of the cells with inhibitors to p38 MAP kinase (SB203580, 
10 μM) and caveolae activity (filipin, 20 μM) markedly suppressed expression of both 
Hsp27 and caveolin-1 in CEE-treated keratinocytes. Taken together, these data indi-
cate that activation of p38 MAP kinase contributes to regulating Hsp27- 
and caveolae-mediated actin dynamics. Upregulation of these signaling pathways 
is likely to play an important role in the mechanisms of vesicant-induced skin toxic-
ity. Supported by CA100994, CA093798, ES004738, ES005022, GM034310 and 
AR055073.

**PL 62**

**THE VESICANT 2-CHLOROETHYL ETHYL SULFIDE (CEES) INDUCES EXPRESSION OF PROLIFICATIVE MARKERS IN A FULL-THICKNESS HUMAN SKIN EQUIVALENT.**

A. T. Black1, P. H. Havrileski2, A. M. Vetranio1, L. G. Grant1, R. P. Casillas2, D. E. Heck1, D. R. Gerecke1, T. L. Laskin1 and D. L. Laskin1
1 Rutgers University, Piscataway, NJ; 2MateriK Corporation, Ashland, MA; 3UMDNJ-RWJ Medical School, Piscataway, NJ; 4U.S. Court Guard Academy, New London, CT; 5Batelle Biomedical Research Center, Columbus, OH and 6New York Medical College, Valhalla, NY.

The chemical warfare agent sulfur mustard is a potent vesicant that produces in-
flammation, edema and blistering in exposed skin. To assess molecular mechanisms 
mediating these effects we used CEEs, a model sulfur mustard vesicant, and ana-
yzed its effects on Epiderm-FT™ (EFT), a commercially available full-thickness 
human skin equivalent that exhibits similar structure and chemical composition as 
human skin. CEEs treatment (100-1000 μM) caused a time-dependent (2-72 hr) 
increase in pyknotic nuclei and vacuolization in basal keratinocytes. Microvesicles 
were also observed at the dermal-epidermal junction 24-72 hr after exposure. Using 
techniques in immunohistochemistry, we found concentration-dependent increases 
in expression of proliferating cell nuclear antigen (PCNA), a marker for DNA syn-
thesis, COX-2, a marker for inflammation, and poly(ADP-ribose) polymerase 
(PARP), a marker for apoptosis. Maximal levels of PCNA, COX-2 and PARP were 
observed after 6-24 hr. PCNA was expressed in keratinocytes and fibroblasts at all 
time points while PARP was detected primarily in keratinocytes. In contrast, COX-
2 was expressed in fibroblasts at all time points and was detected in keratinocytes 
only at the 6 hr time point. FACS array analysis of the culture medium from the 
EFT tissues showed that CEEs also induced production of the proinflammatory 
cytokines IL-6 and IL-8 after 24 hr. These data indicate that CEEs induces an early 
inflammatory response which is followed by tissue damage and apoptosis in both 
the epidermal and dermal layers. These findings suggest that the EFT skin equiva-
 lent is a useful in vitro model for characterization of the biological effects of vesi-
cants on the skin. Supported by CA100994, CA093798, ES004738, ES005022, GM034310 and AR055073.

**PL 63**

**LATE PHASE BIOMARKERS OF SULFUR MUSTARD-INDUCED INJURY.**

J. Seagrave1, L. Blair, G. Grotendorst, L. Herrera, M. Lehman, T. March and W. Weber
1 Lovelace Respiratory Research Institute, Albuquerque, NM.

Inhalation exposure to sulfur mustard presents a potential chemical threat. In addi-
tion to well-known vesicant effects on skin, it also causes rapid myelosuppression 
and long-term effects. In the present study, a superficial dermal skin lesion was es-
lished in BALB/c mice and exposed directly to CEES, a model sulfur mustard vesicant. CEES treatment (100-
1000μM) caused a concentration-dependent increase in actin polymerization in 
keratinocytes labeled with rhodamine-phalloidin and analyzed by confocal microscopy. CEE also upregulated Hsp27 and caveolin-1 expression and activated the 
p38 MAP kinase signaling pathway through increased phosphorylation of p38 MAP kinase. Treatment of the cells with inhibitors to p38 MAP kinase (SB203580, 
10 μM) and caveolae activity (filipin, 20 μM) markedly suppressed expression of both 
Hsp27 and caveolin-1 in CEE-treated keratinocytes. Taken together, these data indi-
icate that activation of p38 MAP kinase contributes to regulating Hsp27- 
and caveolae-mediated actin dynamics. Upregulation of these signaling pathways 
is likely to play an important role in the mechanisms of vesicant-induced skin toxic-
ity. Supported by CA100994, CA093798, ES004738, ES005022, GM034310 and AR055073.

**PL 64**

**LONG-TERM CHARACTERIZATION OF A SUPERFICIAL DERMAL SKIN INJURY FOLLOWING EXPOSURE OF HAIRLESS GUINEA PIGS TO HD VAPOR.**

S. Dachir, M. Cohen, L. Tverva, R. Sahar, H. Gutman, V. Horvitz and T. Kadar
1 Pharmacology, Israel Institute for Biological Research, Rehovot, Israel; 2Rutgers University, New Brunswick, NJ; 3UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

Sulfur mustard (HD) is a potent vesicant warfare agent that causes severe damage to 
the skin. Acute skin exposure to HD may result in long-term medical complica-
tions. Although many years of research in this area, no effective treatment for HD 
skin injury is yet available, therefore, it is of great importance to find a treatment 
that will improve the healing process and ameliorate or even prevent skin damage 
and long-term effects. In the present study, a superficial dermal skin lesion was es-
lished and characterized in the hairless guinea pig (HGP) model. HGP skin was 
exposed to HD vapor for 15 min. Clinical evaluation was conducted using reflectance colorimetry, transdermal water loss and wound area measurements. Prostaglandin (PGE) content and metalloproteinase-2 & 9 (MMP-2, MMP-9) ac-
tivity were measured, as well as histological and immunohistochemical assess-
ment of the extent of the injury were conducted up to four weeks post-exposure. 
Following exposure, typical symptoms of HD skin injury developed including ery-
thema and edema, impairment of skin barrier, ulcers and increase in PGEa and 
MMP-2 & 9. Histological evaluation revealed severe damage to the epithelium and 
upper dermis, vasculitis at the basement membrane zone, significant edema in the 
dermis and infiltration of inflammatory cells. At four weeks after exposure healing 
was not completed: epithelial hyperplasia, inflammatory cells, absence of hair folli-
ces and hemorrhage in the upper dermis were observed. The results indicated that 
the effects of HD vapor on HGP skin are similar to its effects on human skin. 
Therefore, the HGP can be used as an animal model for studying acute as well as 
chronic effects of HD, for conducting long-term follow-up of the healing processes 
and for the evaluation of treatments efficacy. Part of the work was supported by the U.S. Army Medical Research and Material Command, under award #: W81XWH-08-2-0128.

**PL 65**

**SELECTIVE CROSS-LINKING OF THIOREDOXIN REDUCTASE IN LUNG EPITHELIAL CELLS BY NITROGEN MUSTARD, A MODEL SULFUR MUSTARD VESICANT.**

Y. Jay1, D. E. Heck2, R. P. Casillas1, D. L. Laskin1 and L. D. Laskin1
1 Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ; 2Environmental Health, New York Medical College, Valhalla, NY; 3Biomedical Science & Technology, Batelle Biomedical Research Center, Columbus, OH and 4Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.

Oxidative stress plays a critical role in sulfur mustard-induced toxicity. The thio-
redoxin system, which consists of thioreredoxin reductase (TrxR), thioreredoxin, and 
NADPH, is a critical cellular antioxidant that is important in redox regulation and 
protection against oxidative stress. Nitrogen mustards, including mechlorethamine (HN2), contain two electrophilic chloroethyl side chains which can readily react 
with nucleophilic amino acids in proteins, a process that can lead to changes in pro-
tein structure and/or function. Previously, we reported that the monofunctional vesic-
ant 2-chloroethyl sulide targets TrxR by selectively alkylating selenocysteine in the 
C-terminal redox motif of the enzyme, a process leading to enzyme inhibi-
tion. In the present studies, we evaluated the effect of HN2 on the thioreredoxin sys-
tem using A549 lung epithelial cells and purified TrxR. HN2 was found to cause a 
concentration-dependent (1-10 μM) inhibition of TrxR in both systems. Western 
blot analysis revealed decreases in the TrxR monomer and simultaneous increases in 
TrxR dimer formation. Using biotin-conjugated iodoacetamide (BIA) to selec-
tively react with low pKa seleno or thiol groups on proteins at pH 6.5, we found that 
HN2 differentially decreased BIA-labeled TrxR in A549 cells and with puri-
fied enzyme, suggesting a decrease in the reduced form of TrxR. These results sug-
gest that HN2 inactivates TrxR by targeting seleno and/or thiol containing redox 
centers and cross-linking TrxR peptides. Disruption of the Trx system is likely to
contribute to vesicant-induced cytotoxicity. Supported by NIH grants AR055073, CA03798, CA109994, CA132624, ES009478, ES050522, ES017389, and GM034310.

**PL 66** DIFFERENT METHODS TO STUDY THE GENOTOXIC MARKER γH2AX FOLLOWING SULFUR MUSTARD EXPOSURE IN CULTURED HUMAN SKIN CELLS AND A SKIN TISSUE CONSTRUCT.


Sulfur mustard (2,2'-dichlorodieethyl sulfide, SM) is a cytotoxic chemical warfare agent. The skin serves as a principal target site for in vivo toxicity of SM exposure resulting in the formation of blisters and inflammation. To elucidate genotoxic effects of SM, normal human epidermal keratinocytes (NHEK, Lonza Corp., MD) and a commercially available, multicellular skin tissue construct, EpiDerm™ (MadTek Corp., MA), served as in vitro models to observe the presence of γH2AX foci. γH2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double stranded DNA break sites. In its phosphorylated state, γH2AX can be used as a reproducible indicator of genotoxic injury. Three different methods were used to confirm the presence of γH2AX and its occurrence over a dose and time range. Cells and constructs were exposed to 0, 50, 100 or 300 μM concentrations of SM for 2 or 24 hrs. Following exposure, tissues underwent fluorescent immunohistochemistry to stain for γH2AX while cellular activity was measured by flow cytometry and western blotting. Preliminary data shows that SM exposure results in the formation of γH2AX, which is prevalent at 300 μM SM. This indicator of DNA damage will be a useful biomarker for the study of SM toxicity and potential therapeutic compounds. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

**PL 67** PLASMA MEMBRANE-BOUND OXIDOREDUCTASES ARE AN IMMEDIATE TARGET OF NITROGEN MUSTARD IN PULMONARY EPITHELIAL CELLS.

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Pulmonary exposure to the chemical warfare agent sulfur mustard results in delayed epithelial necrosis in vivo. Although the pathology of cellular necrosis and apoptosis following exposure to sulfur mustard is well characterized, the initiating events have not been determined. Proteins at the cell surface are likely targets for vesicating agents because vesicants are highly chemically reactive and many are lipid soluble. Oxidoreductases at the cellular plasma membrane are important for the oxidation of cellular reducing equivalents (NADH and NAPDH) which are the results of cell metabolism and for the prevention of intracellular reductive stress. These enzymes reduce extracellular substrates including molecular oxygen and plasma-bound ubiquinones. We found that exposure of A549 pulmonary epithelial cells to the nitrogen mustard chloroethyl methylhydrochloride (100 μM), a sulfur mustard analog, resulted in an immediate, dose-dependent decrease in activity of cell surface oxidoreductases. Maximum inhibition was achieved after 15 minutes of exposure. Cyanide stimulates reductive stress by inhibiting mitochondrial respiration. This prevents the cytoplasmic conversion of NADH and leads to the alternative pathway of NADH reoxidation through enhanced plasma membrane oxidoreductase activity. Nitrogen mustard reduced cyanide-stimulated oxidoreductase activity in a dose-dependent manner. However, nitrogen mustard failed to affect cellular respiration as long as seven hours following exposure, suggesting that mitochondrial function was not an immediate target of vesicants. These findings suggest that inhibition of plasma membrane reductases may be a triggering event in the pathology of mustard toxicity and may contribute to the toxicity of vesicating agents through induction of intracellular reductive stress.

**PL 68** SULFUR MUSTARD VAPOR DEPOSITION, TISSUE DISTRIBUTION AND CLEARANCE IN THE HAIRLESS GUINEA PIG.


Sulfur Mustard (SM) is a vesicant (blistering agent) that targets lung, skin, eye, and testes. SM was used as a chemical warfare agent during World War I and, more recently by Iraqi troops against Iranians during the Iran-Iraq war. We have developed an animal model of dermal exposure to hairless guinea pigs. In this model, animals exposed to ~500 mg/m3 SM vapor over three 1 cm diameter sites on their backs for 6 or 12 minutes experience local inflammation, tissue degeneration and necrosis, fluid dysregulation, and hyperplastic changes. Lesions occur in a dose-dependent manner and persist in the high dose group through 14 days post exposure. The purpose of this study was to examine the extent of vapor deposition, tissue distribution, and elimination of SM following dermal exposure. Anesthetized male hairless guinea pigs were exposed on 3, 1-cm sites for 12 minutes to 14C-SM (1.2 nCi/g SM). Groups of three guinea pigs were euthanized at 0.5, 2, 4, 8, 12, 24, and 168 hours post exposure. The 168 hour time point animals were placed in metabolic cages for excreta collection. At 0.5 hr post exposure, 9.07 ± 0.91 μg SM equivalents (mean ± SD; n = 3; 10.9 ± 1.09 μg) were present in skin samples. At 168 hr, 0.51 ± 0.64 μg SM equivalents remained at the exposed sites. SM equivalents were first detected in blood at 2 hr post exposure (87 ± 14 ng SM equivalents/g), with 26 – 72 ng/g still present at 168 hr. SM equivalents in kidney peaked at 2 hr post exposure (1.51 ± 0.31 μg/kg) and persisted through 8 hr. SM equivalents were detected in urine throughout 168 hr post exposure, likely in the form of a thioglycol. Results indicate that robust and persistent SM induced dermal lesions can be induced in the hairless guinea pig by deposition of approximately 11 μg/cm2 skin. Further, SM adducts may circulate in blood up to 7 days post exposure. Although SM equivalents are present in kidney, to date no lesions have been identified in this organ. Research funded under U54 NS058185-01.

**PL 69** THERAPEUTIC EFFICACY OF SILIBININ, A NATURAL FLAVANONE, IN SULFUR MUSTARD ANALOG INDUCED SKIN TOXICITY.

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Sulfur mustard (HD), a chemical warfare agent, inflicts devastating and extended injurious effects on skin. Effective therapeutic interventions against HD-caused skin injury are deficient due to incomplete knowledge of the related cellular mechanisms, which is mainly attributed to the requirement of efficient and more applicable skin injury animal models. Our recent studies have elaborated on the mechanisms involved in HD analog 2-chloroethyl ethyl sulfide (CEES)-induced skin injury, and have established CEES-induced injury biomarkers in mouse skin cells and SKH-1 hairless mouse. Employing these efficient CEES-induced skin injury models, we are presently conducting efficacy studies using silibinin, a natural flavanone with proven antioxidant, anti-inflammatory and anti-cancer properties in skin and other tissues. In our in vitro studies with mouse epidermal JB6 cells, 10 μM silibinin treatment 1-15 min post-CEES exposure resulted in significant (p<0.05) reversal of CEES-induced reduction in DNA synthesis and DNA damage (H2AX and p53 activation). In mouse skin fibroblasts, 10 μM silibinin treatment for up to 30 and/or 60 min post-CEES exposure caused a significant (p<0.05) reversal of CEES-induced a) reduction in cell viability, b) cell death especially apoptotic death, and c) DNA damage in terms of DNA tail extent moment in comet assay as well as H2AX and p53 activation. Efficacy study with 1-5 mg doses of silibinin treated topically on SKH-1 hairless mice 30 min post CEES exposure, demonstrated a significant (p<0.05) reversal in CEES-caused increases in skin fold thickness, and myeloperoxidase levels indicating neutrophil infiltration in CEES-treated skin tissue in a countermeasure setting. Studies are underway to determine the effect of silibinin on other established biomarkers in both cell culture and mouse skin models, which will facilitate to identify and define efficacy of silibinin and antioxidant therapy as effective countermeasure against HD-caused skin injury.

**PL 70** GENE EXPRESSION ALTERATIONS IN IMMUNE SYSTEM PATHWAYS FOLLOWING EXPOSURE TO IMMUNOSUPPRESSIVE CHEMICALS.

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Diethylstilbestrol (DES), Desamzenoxane (DCE), Cyclophosphamide (CP), and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) are immunosuppressive chemicals that induce similar pathophysiological endpoints in the thymus but have more diverse effects in the spleen. This study examined transcriptional changes in the thymus and spleen of female B6C3F1 mice following exposure to these agents to assess possible mechanisms of action. RNA was analyzed using Illumina Sentrix™ Array Matrix and Ingenuity Pathway Analysis. Immune tests were conducted to anchor the changes in gene expression. All four chemicals induced thymic atrophy, and altered the relative proportion and absolute number of thymocyte and splenocyte subpopulations. DEX and CPs exposure led to atrophy in the spleen, while DES...
exposure resulted in splenomegaly. Mixed lymphocyte responses, and basal and anti-CD3 antibody-mediated splenocyte proliferation were decreased following treatment with DEX, DES, and CPS, but not TCDD. The most notable transcriptional effect of exposure to DEX, DES, or CPS in thymus was modulation of the T-cell Receptor Signaling pathway, which plays a role in the development and function of T-cells. Ironically, only DES regulated this pathway in spleen. Upregulation of genes associated with the Antigen Presentation and Dendritic Cell Maturation pathways were the most distinctive effects of TCDD exposure in thymus. These elements, which were also upregulated by DEX and DES, contribute to positive and negative selection. While changes in gene expression in thymus showed many commonalities between chemicals, in the spleen the gene expression profiles were quite distinct for each chemical. DES altered expression in many pathways associated with T- and B-cell function and immune cell signaling, but the effects of DEX, CPS, and TCDD were more limited. These findings may provide insight into the mechanisms invoked by each chemical and into the different action that a single chemical has in two lymphoid organs.

**PL 71**

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

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental toxin known to inhibit immunoglobulin (Ig) gene expression in animal studies. Transcriptional regulation of the Ig heavy chain (IgH) involves the 3’IgH regulatory region (3’IgHRR) and its enhancers (hs3, hs1,2, and hs4), which contain DNA binding sites for several transcription factors, including NF-κB and dioxin response elements (DRE). TCDD binds inducing the aryl hydrocarbon receptor (AhR) complex to a DRE in both the hs1,2 and hs enhancers and inhibits murine 3’IgHRR activation in a well characterized mouse B-cell line (CH12.LX). In humans, a polymorphism of the hs1,2 enhancer, resulting in varying numbers of a 53 bp sequence tandemly repeated, has been correlated with autoimmune diseases like IgA nephropathy and Celiac disease. The repeated sequence contains Kβ and DRE binding sites. The objective of this study was to comparatively evaluate the effect of TCDD and LPS on human and mouse hs1,2 enhancers in the CH12.LX model. In transient luciferase studies, an increased number of repeats in the human hs1,2 enhancer increased the sensitivity to LPS. Interestingly, TCDD also markedly enhanced human hs1,2 activity and even augmented LPS-induced activation. TCDD-induced activation positively correlated with the number of repeats. This starkly contrasted with TCDD-induced inhibition of the mouse hs1,2 and 3’IgHRR in LPS-stimulated CH12.LX cells. Through sequence analyses, we verified that the human hs1,2 enhancer retains the Kβ and DRE binding sites but lacks binding sites for B-cell specific activator protein and NF-κB which are both important to mouse hs1,2 regulation. Mutational analyses are underway to evaluate the significance of these binding sites in TCDD-induced modulation of both the human and mouse hs1,2 enhancer. Since TCDD represents a large class of chemicals found in the environment, diet, and pharmaceuticals, understanding chemical-induced modulation of the 3'IgHRR enhancers may provide a clue to the etiology of certain autoimmune diseases. (Supported by NIEHS R01ES014676)

**PL 72**

THE ROLE OF AHR IN MATURATION OF DENDRITIC CELLS.

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Genes important for dendritic cell (DC) activation and maturation such as chemokines and phenotypic surface markers were investigated in this study. In addition to being functionally defined, many of these genes are highly expressed in mature DC or show significant changes in expression during cell differentiation. Using the AHR antagonist MFO and prototypic AHR agonists TCDD, we examined whether AHR activity would affect the differentiation and activation of human DC derived from U937 cells into mature DC phenotype. Expression of CD86 mRNA was clearly increased by TCDD. In contrast, MFO suppressed the expression of CD86 in differentiating DC. In parallel, the expression of CD1a was augmented by TCDD and suppressed by MFO. Further, we tested the effect of MFO and TCDD on the expression of the DC-specific genes DC-CIK, IL-8 and DC-STAMP. As described earlier in macrophages, TCDD increases IL-8 expression about 20-fold in U937 derived DC. DC-STAMP was less but also significantly increased by TCDD. In contrast, the upregulation of DC-CIK was clearly blocked in presence of TCDD. As in the case of DC surface markers, MFO has a contrary effect compared to TCDD by increasing DC-CIK, but suppressing IL-8 and DC-STAMP. Data from experiments with bone-marrow derived DC (BM-DC) from mice confirm the results received from human DC models showing a significant increase of CD86 and suppression of DC-CIK in differentiating DC from wild type but not from AHR null mice by TCDD. Furthermore, the constitutive expression of chemokine receptor CCR6 was decreased in AHR null mice compared to wild type mice and further increased by TCDD in DC only from wild type mice. The expression of CCR6 is associated with a newly identified AHR-dependent IL-22-producing helper T cell population. These data suggest a critical role of the AHR in the regulation of DC-specific surface markers and chemokines which are critical for regulation of T cell differentiation and support the hypothesis that the AHR exerts a physiological role in DC differentiation and activation.

**PL 73**

DIRECT REGULATION OF BACH2 BY 2, 3, 7, 8- TETRACHLORODIBENZO-P-DIOXIN IN MURINE B LYMPHOMA CH12.LX CELLS.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is an environmental contaminant known to alter B cell function, resulting in marked suppression of the primary immune response. The molecular mechanisms responsible for these immunotoxic effects involve transcriptional regulation through the aryl hydrocarbon receptor (AHR). To identify the novel genes that are directly modulated by the ligand-activated AHR during B cell differentiation we performed a genome wide localization analysis or ChIP on chip. For the ChIP on chip, DNA from the mouse B cell line, CH12.LX was used, in which comparisons were made between CH12.LX cells activated with lipopolysaccharide in the presence or absence of TCDD for 60 min. One of the genes identified in this screen was Bach2, which is a B cell specific transcriptional repressor. It is known to repress Blimp1 which is a master regulator of the B cell differentiation program. To further elucidate the putative involvement of Bach2 in TCDD-mediated impairment of B cell differentiation and function, a time course performed to assess the effects of TCDD on Bach2 mRNA levels in LPS-activated CH12.LX cells by quantitative Real-Time PCR. These studies showed that in LPS-activated CH12.LX cells, Bach2 mRNA levels remained unchanged in naïve cells at 2h, then decreased at 4h and remained decreased through a 24h time period. In contrast, TCDD-treated cells exhibited a strong induction of Bach2 at 2h, which then decreased to the same levels as that in LPS-activated cells. In additional studies, TCDD induced a concentration-dependent increase in Bach2 mRNA levels at the 2h time point. Collectively, these studies suggest that Bach2 is directly regulated by AHR and may be responsible for impaired Blimp-1 regulation and altered B cell differentiation by TCDD. (Supported in part by NIH P42 ES04911 and R01 ES05240)

**PL 74**

ALLERGEN-INDUCED CHANGES IN INTERLEUKIN-17 EXPRESSION IN MICE.

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Polarized subsets of T helper (Th) cells that are characterized by selective cytokine secretion patterns orchestrate the development of immune and allergic responses. It has been demonstrated previously that prolonged topical (13 day) exposure of BALB/c strain mice to different classes of chemical allergen preferentially activates divergent Th cell subsets. Thus, the contact allergen 2,4-dinitrochlorobenzene (DNCB) and the respiratory sensitizer trimellitic anhydride (TMA) stimulate Th1 and Th2 cells, respectively. Recently a further subset of inflammatory Th cells that secretes IL-17 is being shown to play important roles in some autoimmune and inflammatory conditions. In the current investigations, the expression of IL-17 isoforms has been examined in the skin and lymph nodes following topical exposure to chemical allergens. BALB/c strain mice were exposed to a single topical dose of either 1% DNCB or 25% TMA, or to vehicle alone, for 30 min to 72 h. At various times thereafter, cytokine production by skin explants (prepared from dorsal halves of ear tissue) or by draining auricular lymph node cells (LNC) was measured by cytokine secretion patterns orchestrate the development of immune and allergic responses. It has been demonstrated previously that prolonged topical (13 day) exposure of BALB/c strain mice to different classes of chemical allergen preferentially activates divergent Th cell subsets. Thus, the contact allergen 2,4-dinitrochlorobenzene (DNCB) and the respiratory sensitizer trimellitic anhydride (TMA) stimulate Th1 and Th2 cells, respectively. Recently a further subset of inflammatory Th cells that secretes IL-17 is being shown to play important roles in some autoimmune and inflammatory conditions. In the current investigations, the expression of IL-17 isoforms has been examined in the skin and lymph nodes following topical exposure to chemical allergens. BALB/c strain mice were exposed to a single topical dose of either 1% DNCB or 25% TMA, or to vehicle alone, for 30 min to 72 h. At various times thereafter, cytokine production by skin explants (prepared from dorsal halves of ear tissue) or by draining auricular lymph node cells (LNC) was measured by cyto- kinne-specific enzyme-linked immunosorbant assay (ELISA). Topical treatment with DNCB, but not to TMA or vehicle alone, provoked a rapid increase in IL-17A and IL-17F isoforms, peaking at 3 h. Levels of IL-17F were generally some 5-fold higher than those observed for IL-17A. DNCB-activated LNC also produced high levels of both IL-17A and IL-17F, reaching maximal levels after 6h of exposure. The heterodimer (IL-17A/F) was also detected, but at considerably lower levels. Treatment with TMA induced the same pattern of cytokines in the draining lymph node, but with slightly delayed kinetics, peaking at 48 to 72h. These data

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demonstrate that chemical allergen provokes the rapid production of IL-17 isoforms in the skin and draining lymph node which may play a role in the acquisition of sensitization.

**SKIN TYPE 2 CYTOKINES AND SERUM IGE ANTIBODY LEVELS ARE INCREASED BY ALLERGEN IN AGING MICE.**

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It has been reported that ageing is associated with impairment of the skin immune system, with an increased incidence of both cutaneous infection and malignancy in the elderly. We have observed previously that in both human and murine skin, ageing is associated with reduced frequency of epidermal Langerhans' cells (LC), and that LC migration in response to interleukin (IL)-1β-dependent stimuli is markedly reduced. In the current investigations we have examined the impact of age on the quality of induced immune responses in mice, measured as a function of IgE antibody production and cytokine expression following topical exposure to a type 2 inducing chemical sensitizer, the respiratory allergen trimellitic anhydride (TMA). Young (6-8 week old) and aged (≥30 week old) female BALB/c strain mice (n=5 to 10) received repeated topical applications of 10% TMA over a 13 day period. Serum was analyzed for total IgE concentration by sandwich enzyme-linked immunosorbant assay (ELISA) and cytokine production by skin explants (prepared from dorsal halves of ear tissue) was measured by protein array (Luminex). Exposure to TMA stimulated increases in the total serum IgE concentration in both aged (97% ± 145 ng/ml) and young (367 ± 47 ng/ml) mice compared with AOO treated controls (202 ± 42 and 145 ± 29 ng/ml, in aged and young mice, respectively). This represents a 5-fold and 2.5-fold allergen-induced increase in total serum IgE in aged mice compared with their younger counterparts. In addition, more vigorous type 2 cytokine expression was recorded in allergen-treated skin of aged mice than that observed in young skin. Thus, higher levels of IL-4, IL-10 and IL-13 were observed whereas similar levels of IL-12 were recorded. The immune system in aged mice is apparently more skewed towards a type 2 response than in their younger counterparts, which may be related to recent reports of age-related changes in regulatory T (Treg) cell numbers and function in this mouse strain. These data suggest that age may be one predisposing factor in the acquisition of some forms of chemical allergy.

**PERINATAL BISPHENOL A EXPOSURE ALTERS B LYMPHOPOIESIS AND CYTOKINE/CHEMOKINE PRODUCTION IN ADULT C57B6/129 MALE MICE.**

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Humans are unavoidably exposed to bisphenol A (BPA), in some cases at levels that cause altered endocrine function in laboratory rodents. The developing immune system is also highly sensitive to environmental disruption of chemicals such as BPA. In the present study, C57B6/129 mice were exposed to 1 mg/kg BPA by maternal intraperitoneal injection, from gestation day 9 until end of lactation. Progenitor B cells and total B cells were significantly decreased in the bone marrow of BPA mice at 20 weeks of age, suggesting a permanent effect on B lymphopoiesis. Serum from the BPA mice showed an increase in basal levels of the pro-inflammatory cytokines G-CSF, GM-CSF and IL-1β. Con-A stimulated splenocytes from these mice produced significantly more IL-12, whereas similar levels of IL-12 were recorded. The immune system in aged mice is apparently more skewed towards a type 2 response than in their younger counterparts, which may be related to recent reports of age-related changes in regulatory T (Treg) cell numbers and function in this mouse strain. These data suggest that age may be one predisposing factor in the acquisition of some forms of chemical allergy.

**EVALUATING THE EFFECT OF TYROSINE KINASE INHIBITORS ON MITOCHONDRIAL FUNCTION IN RAT PRIMARY CARDIOMYOCYTES.**


The tyrosine kinase inhibitor imatinib (Gleevec) has been associated with a very low incidence of clinical cardiac injury resulting in debate over the drug’s link to injury. No cardiotoxicity was observed in preclinical studies but Kerkela et al (2006) demonstrated morphological changes in mitochondria from both mice and human treated with imatinib suggesting a role for mitochondrial dysfunction. Additionally, in vitro studies in cardiomyocytes demonstrated that imatinib induced ER stress and mitochondrial dysfunction as measured by changes in mitochondrial membrane potential. This effect was circumvented by expressing a mutant form of c-ABL. Fernández et al (2007) reengineered Imatinib to remove c-ABL and JNK activity and the reengineered analog, WBZ-4, had no effect on ventricular ejection volume in mice.
We investigated the effect of imatinib and WBZ_4 on mitochondrial function by directly measuring oxygen consumption and media acidification using the Seahorse XF24 in neonatal rat ventricular myocytes (NRVM). The data demonstrated that both imatinib and WBZ_4 had similar effects in vitro. Both compounds were moderately cytotoxic in NRVM, and altered mitochondrial function, albeit at higher concentrations than previously reported. At sub-cytotoxic concentrations, the compounds increased oxygen consumption, and inhibited the ability of cells to respond to uncoupling. Cellular Systems Biology studies using Cellumen’s CellCiphr™ high content imaging confirmed that both compounds altered mitochondrial membrane potential and generated reactive oxygen species. Together, these results affirm the previously reported mitochondrial effects of imatinib but demonstrate that the inactive analog WBZ_4 has similar mitochondrial effects. Therefore, the data suggest that the mechanism of cardiotoxicity for imatinib may not be due solely to mitochondrial dysfunction since both compounds show similar profiles in vitro, yet with disparate effects on the heart in vivo.

XANTHINE OXIDASE AND NADPH OXIDASE CONTRIBUTE TO MITOCHONDRIAL IMPAIRMENT INVOLVED IN COCAINE-INDUCED LV DIASTOLIC DYSFUNCTION.

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Recent studies show that long-term cocaine use induces diastolic impairment. This functional effect may be linked to cocaine-triggered oxidative stress. Indeed, previously we have demonstrated that myocardial NADPH and xanthine oxidase (XO) contribute to ROS generation in cocaine-treated rats. We hypothesized that cocaine-induced ROS production could induce mitochondrial damage that in turn might participate in ventricular diastolic dysfunction. Wistar rats were treated with cocaine alone (2x7.5 mg/kg/day, IP) or with a NOX inhibitor (apocynin, 50 mg/kg/day, po) or a XO inhibitor (allopurinol, 50 mg/kg/day, po). These groups were compared to control rats (saline solution, IP). After 7 days, LV pressure-volume signals were acquired. Oxygen consumption was measured in situ on permeabilized cardiac fibers isolated from the LV. ROS production was measured using electron paramagnetic resonance in both subsarcolemmal (SSM) and inter fibrillar (IFM) mitochondria. Our results show that cocaine-induced cardiac dysfunction is characterized by a diastolic impairment. Indeed cocaine induces an increase of TAU, an index of LV relaxation and of end-diastolic pressure volume relation (+80% and +171% respectively, p<0.05). Both apocynin and allopurinol were able to improve ventricular relaxation. Further, we found that cocaine increased oxygen consumption in mitochondria specifically through complex I (+34%, p<0.05) and complex III (+120%, p<0.05) whereas ATP production was decreased. Moreover, ROS level increased only in IFM from cocaine rats (+74%, p<0.05). In contrast, apocynin or allopurinol treatments prevented the rise in ROS levels and prevented the mitochondrial respiratory chain alterations. In conclusion, this work shows that cocaine-induced LV diastolic impairment is associated with a specific mitochondrial dysfunction. An increase in ROS production via NADPH and XO induces a mitochondrial dysfunction which in turn contributes to the development of oxidative stress and ventricular diastolic dysfunction.

Mitochondrial fusion and autophagy aid in removal of persistent mitochondrial DNA damage.

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Mitochondrial DNA (mtDNA) integrity is critical for human health; however, it is unclear how helix-distorting mtDNA adducts formed after exposure to environmentally important genotoxins such as ultraviolet radiation and P4Hs are handled. mtDNA may be particularly susceptible to these genotoxins due to the absence of nucleotide excision repair, the primary repair mechanism for such DNA adducts in nuclear DNA. We investigated the removal of photodimers in mtDNA via mitochondrial fusion, fission and autophagy in Caenorhabditis elegans. Larval fusion, fission and autophagy mutant C. elegans were exposed to serial UVC doses over 48 hours. This exposure protocol allows for accumulation of mtDNA damage without lasting damage to nuclear DNA and results in measurable larval growth arrest. Strains including the mtDNA gene deletion and fusion genes bro-1 and eat-3 exhibited exacerbated larval growth arrest with little to no growth recovery after 72 hours. We concluded that these proteins are required for normal recovery from mtDNA damage-induced larval growth arrest. In order to test directly the contribution of autophagy, fusion and fission proteins to removal of UVC-induced mtDNA damage, we performed RNAi knockdown of autophagy, fusion and fission genes in UVC-treated adult glp-1 C. elegans. Knockdown of autophagy and fusion genes inhibited removal of UVC-induced DNA damage, as measured by a semi-quantitative PCR-based assay. These data suggest that autophagy and fusion processes are involved in the removal of bulky DNA damage in mitochondria. Mitochondrial dysfunction as a result of bulk mtDNA damage may trigger mitochondrial remodeling. Results indicate the relative ATP levels are reduced in UVC treated animals. Therefore, we hypothesize that UVC-induced mtDNA damage is removed via fusion-mediated mitochondrial remodeling and subsequent autophagy, possibly triggered by mitochondrial dysfunction. This research was supported by funding from the NIHES, 1 P30 ES-011961-01A1 and National Institute of Neurological Disorders and Stroke. 1 R21 NS065468-01.

A REAL-TIME NON-INVASIVE 96-WELL PLATFORM FOR INVESTIGATING THE EFFECTS OF DRUGS ON MITOCHONDRIAL RESPIRATION AND GLYCOLYTIC RATES OF INTACT CELLS.

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Compound attrition due to toxicity is a concern for pharmaceutical companies. Mitochondrial impairment has been implicated in the withdrawal of drugs such as tolcapone, troglitazone and cerivastatin. We tested the OCR and ECAR of HepG2 cells and show that the platform had intra- and inter-assay variations of less than 15%. The platform was validated with mitochondrial inhibitors of respiration, an uncoupler of oxidative phosphorylation, and an inhibitor of glycolysis. A selection of drugs was then investigated for their acute effects on the OCR and ECAR of HepG2 cells. For comparative purposes, we also examined the effects of these drugs on respiration of isolated rat liver mitochondria using an oxygen sensitive phosphorescence probe. Tolcapone and Entacapone, used in the treatment of Parkinson’s disease, were uncouplers of respiration in HepG2 cells and caused an increase in the glycolytic rate of these cells. Nilutamide and Flutamide, anti-androgens given in the treatment of prostate cancer, inhibited oxygen consumption of cells and increased their glycolytic rate. The anti-diabetic drugs, Troglitazone and Cigitazone, inhibited respiration whereas Pioglitazone did not. All of the drugs that affected the bioenergetics of cells also impaired oxygen consumption of isolated mitochondria. In summary, the 96-well platform for measuring OCR and ECAR of cells enabled us to investigate the effects of drugs on the bioenergetics of HepG2 cells and can be implemented as part of a screen for identifying drug-induced mitochondrial impairment.

A REAL-TIME NON-INVASIVE 96-WELL PLATFORM FOR INVESTIGATING THE EFFECTS OF DRUGS ON MITOCHONDRIAL RESPIRATION AND GLYCOLYTIC RATES OF INTACT CELLS.

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Compound attrition due to toxicity is a concern for pharmaceutical companies. Mitochondrial impairment has been implicated in the withdrawal of drugs such as tolcapone, troglitazone and cerivastatin. We tested the OCR and ECAR of HepG2 cells and show that the platform had intra- and inter-assay variations of less than 15%. The platform was validated with mitochondrial inhibitors of respiration, an uncoupler of oxidative phosphorylation, and an inhibitor of glycolysis. A selection of drugs was then investigated for their acute effects on the OCR and ECAR of HepG2 cells. For comparative purposes, we also examined the effects of these drugs on respiration of isolated rat liver mitochondria using an oxygen sensitive phosphorescence probe. Tolcapone and Entacapone, used in the treatment of Parkinson’s disease, were uncouplers of respiration in HepG2 cells and caused an increase in the glycolytic rate of these cells. Nilutamide and Flutamide, anti-androgens given in the treatment of prostate cancer, inhibited oxygen consumption of cells and increased their glycolytic rate. The anti-diabetic drugs, Troglitazone and Cigitazone, inhibited respiration whereas Pioglitazone did not. All of the drugs that affected the bioenergetics of cells also impaired oxygen consumption of isolated mitochondria. In summary, the 96-well platform for measuring OCR and ECAR of cells enabled us to investigate the effects of drugs on the bioenergetics of HepG2 cells and can be implemented as part of a screen for identifying drug-induced mitochondrial impairment.

MITOCHONDRIAL FUSION AND AUTOPHAGY AID IN REMOVAL OF PERSISTENT MITOCHONDRIAL DNA DAMAGE.

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Antibiotic bacterial drugs comprise a class of considerable structural diversity which can be further divided into families of similar efficacy, selectivity and toxicity. New family members have been developed with increased selectivity and reduced toxicity, but the clinical liabilities of the class have been well-documented, necessitating detailed assessment of potential risk. For example, Penicillins can elicit serious allergic reactions and subclinical liver injury. Macrolides elicit hyper-sensitivity and overt hepatic injury. Tetracyclines, a member of the quinolone family, was withdrawn for hepatic injury. At Pfizer we have previously tested multiple anti-bacterial families in a simple 72 hour cytotoxicity assay and found them all to be negative. Cellumen’s CSB approach utilizes a panel of functional assays to measure the response of 8-10 cellular features at multiple doses and time points: 1, 24 and 48 hrs (rat hepatocytes) or 72 hrs (HepG2). The features report on a set of toxicity mechanisms, such as: oxidative stress, DNA damage, cell cycle modulation, lipid accumulation and mitochondrial function. We determined that families of antibiotics share common activity profiles that reflect different toxicity mechanisms. For example, macrolides are associated with ER stress, steatosis and cell cycle modulation. The polyketides have profound effects on mitochondria, ER stress and DNA damage. The quinolones exhibited profound effects on mitochondrial function. Some members of the quinolone family induced an increase in mitochondrial mass, which may be indicative of a compensatory response. This effect on mitochondrial number was only observed in the quinolone family. These Cellular Systems Biology assays enable an understanding of how molecular structure relates to both the mechanisms of anti-bacterial function as well as off-target toxicity.
TCDD-MEDIATED GENE EXPRESSION PROFILING OF NUCLEATED ENCODED MITOCHONDRIAL GENES INVOLVED IN OXIDATIVE PHOSPHORYLATION.

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Generation of mitochondrial reactive oxygen species (ROS) can be perturbed following exposure to environmental chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Reports indicate that the aryl hydrocarbon receptor (AhR) mediates TCDD-induced sustained hepatic oxidative stress by decreasing hepatic ATP levels, increasing mitochondrial glutathione levels and through hyperpolarization of the mitochondrial inner membrane. To further elucidate the effects of TCDD on the mitochondrial, high-throughput quantitative real-time PCR (htrQRTPCR) was used to evaluate the expression of 103 genes encoding proteins involved in electron transport, oxidative phosphorylation, uncoupling and associated chaperones. Htr-QRTPCR analysis of time course (30 μg/kg TCDD at 2, 4, 8, 12, 18, 24, 72 and 168 hrs) liver samples obtained from orally gavaged immature, ovariectomized C57BL/6 mice identified 60 temporally dysregulated genes (fold change>1.5 and P-value ≤0.1). Dose response studies (0.03 to 300 μg/kg TCDD at 4, 24 and 72 hrs) were subsequently conducted to identify 8 genes. One gene represented electron transport chain (ETC) complex I (NADH dehydrogenase), complex III (cytochrome c reductase), IV (cytochrome c oxidase) and a chaperone, respectively while two genes coded uncoupling proteins and complex V (F0F1 ATPase) subunits, respectively. In contrast, transcript levels of ETC complex II, the succinate dehydrogenase remained unchanged. Putative dioxin response elements were found in the promoter regions of the 8 identified dose-responsive genes. These data suggest that TCDD can affect gene expression associated with mitochondrial function which may contribute to TCDD-induced mitochondrial toxicity. This work was funded by NIEHS SBIR P42ES04911.

DEVELOPMENT OF A SCREENING ASSAY TO IDENTIFY TERATOGENIC AND EMBRYOTOXIC CHEMICALS OR DRUGS USING THE ZEBRAFISH EMBRYO.


The development and validation of novel alternatives for embryotoxicity and teratogenicity of chemicals and drugs in order to reduce animal testing is gaining great interest. The purpose of our research project is to investigate zebrafish embryos and larvae as a simple and fast high-throughput system to predict morphological disorders and lethality during early development. Teratogenic (retinoic acid, valproic acid, caffeine, lithium chloride, iron oxide, indomethacin, methotrexate, thalidomide, methimazole, boric acid) and non-teratogenic (saccharin, D-glucose, sodium cyclamaat, penicillin G, diphenhydramine, aspirin) compounds have been selected and were evaluated using an in-house developed protocol (Selderslaghs et al., 2009, Rep. Tox.). Embryos (12) were exposed within 2 hours post fertilization (hpf) to different concentrations of a compound. The assay then included the time-related evaluation of presence and development of morphological endpoints (somites, otoliths, eyes, tail detachment, heart beat, blood circulation and spinal cord) at embryonic and larval stages up to 144 hpf. Each individual was given a score of 0 (normal) or 1 (abnormal) for both lethality and malformation. Scores were converted to effect percentages for each concentration tested and concentration-response curves were created. LC50 and EC50 were calculated, as well as the teratogenic index (TI = LC50/EC50). Compounds for which a TI > 1 was obtained, were considered to be teratogenic. The results obtained with this zebrafish assay for compounds listed, allow to classify compounds as either developmental toxicants or non-tocxicants. Based on TI-values compounds can be ranked according to their teratogenic potency. Through comparison of results with available mammalian and/or human data, an overall concordance of 81% was obtained, demonstrating the validity of this screening test for teratogenicity. Hence, we propose that this assay can be integrated into screening programs for hazard identification of compounds.

THE EFFECT OF MEDICAL DEVICE COMPOSITION ON RESULTS OF CYTOTOXICITY EVALUATED BY THE DIRECT CONTACT AND ELUTION COLONY ASSAYS.

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The colony assay using V79 cells, is a sensitive in-vitro test evaluating the potential for medical devices to cause cytotoxicity either by direct contact with plated cells or via cells exposed to an extract of a device prepared in culture media. We evaluated the effect of contact lenses made of silicone and non-silicone materials including etafilcon A, balafilcon A, lotrafilcon B, comfilcon A, galyficon A and senofilcon A on cytotoxicity, assessed by number of viable colonies. When cells were seeded on the top of contact lenses, the average colony formation was 63%, for all lenses with exception of lotrafilcon B and comfilcon A which showed a 91% and 14%, respectively. In contrast, when lenses were placed on the top of preplated cells, the colony formation was 90% for all lenses and 14% for comfilcon A. Only the surface of lotrafilcon B supported cell attachment whereas positioning of other lenses in a 24-
well plate prevented free cell movement and attachment to the plate explaining the decrease in colony formation. We investigated the effect of extracts, prepared by incubating lenses in culture media containing 5% FBS for 24 hr, on cytotoxicity. Only extract of comfilcon A prevented colony formation, whereas 100% comfilcon A were formed with extracts of other materials. We investigated whether selective deletion of culture media proteins by comfilcon A and not the potential toxic leakables could explain the cytotoxic effect. Supplementation of comfilcon A extract with fresh serum, extraction in serum-free or high-serum media prevented the cytotoxic effect. Soaking of comfilcon A in a solution of albumin prior to extraction also prevented the cytotoxic effect, suggesting that lens was saturated with proteins and unable to further deplete culture media. In conclusion, to avoid variability and false-positive results in a direct contact assay, placing device on the surface of plated cells as well as a confirmation tests using a 24 hr extraction in serum-free medium or albumin saturation are recommended.

89 PROTECTIVE RESPONSE OF THE AH RECEPTOR TO ANTI-INDUCED BILIARY EPITHELIAL CELL TOXICITY IN SEE-THROUGH MEDAKA.

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The adaptive role of the aryl hydrocarbon receptor (Ah receptor or AHR) in protecting against disease-related conditions remains unclear in nonmammalian mod- els. Therefore, this study focused on the potential role of AHR in response to biliary epithelial cell toxicity and hepatobiliary alteration in medaka. See-through medaka (STII strain) were exposed for 96 h using the biliary toxicant alpha-naphthylhydroxybiocyanate (ANIT) as a reagent, and fish were evaluated daily using histological and ultrastructural analysis, and by imaging directly through the body wall of living fish. Brightfield and transmission electron microscopy showed that a single ANIT dose (40 mg/kg) specifically induced swelling and apoptosis of bile ductular epithelial cells (BPDECs) as early as 6 h after initial exposure. Following ANIT-induced BPDEC toxicity, in vivo imaging of STII medaka showed significant gallbladder discoloration from 48-72 h. Collectively, these pathologic data suggested that ANIT exposure resulted in acute hepatobiliary changes, lasting <96 h following initial exposure. We then tested the potential role of AHR in response to ANIT-induced hepatobiliary alteration. Overall, we demonstrated that (1) transient AHR activation and cytochrome P450 1A (CYP1A) induction in livers occurred during ANIT-induced hepatobiliary impairment, (2) pretreatment with an AHR agonist partially protected against acute hepatobiliary alteration, and (3) using a luciferase-based reporter assay, the bile pigment bilirubin weakly activated mouse AHR and binding to medaka-specific CYP1A promoter, resulting in AHR-driven transcription. Given that bile acids and pigments are present in mammalian and fish liver, these studies collectively suggest that bile-induced AHR activation may be conserved between teleosts and rodents.

90 BIOMARKER FINGERPRINTING OF HEAVY METAL TOXICITY.

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A convergence of technological breakthroughs in the past decade has facilitated search efforts toward development of rapid screening tools for biomarkers of toxicant exposure and effect. Platforms offering genome-wide assessment of an organism’s net response to toxicants are especially attractive. Presently, we exposed cohorts of 25 male zebrafish to concentrations of NiCl2, CdCl2, and Na2Cr2O7, corresponding to their respective 96 hr LC50, LC20 and LC60 for 24 hrs followed by gene expression profiling at both organ and organism level via a custom zebrafish microarray. Parallel histopathology on a small subset of metal-exposed zebrafish enabled phenotypic transcriptional alterations. We report differential gene expression in response to metal exposures in whole zebrafish as well as liver samples. Many of these gene targets link molecular pathways associated with known toxicological responses to metal poisoning, including oxidative stress, cell cycle regulation and immune response signaling. Comparative analysis identified subsets of differentially altered transcripts both overlapping and unique to each metal. We conclude that whole organism gene profiling in zebrafish constitutes a high-throughput yet cost-effective approach to biomarker discovery, particularly since it provides a physiologically relevant context of organism-wide response to toxicants.

91 DEVELOPMENT OF AN IN VITRO ASSAY FOR THE ASSESSMENT OF PHOTOSENSITIZERS.


Photosensitivity is a delayed type IV hypersensitivity induced by a broad spectrum of active components and concurrent exposure of skin to ultraviolet radiation. Due to a high frequency of applied substances in toiletry and clinical therapy there is need for predictive methods in order to determine/classify potential photosensitizers before applying them to cosmetics or pharmaceuticals. Up to now, no adequate in vitro alternatives are available. Our intention was to provide an in vitro photosensitivity assay for assessing photosensitizers. Since denticrines play a key role in the induction of contact allergies this in vitro assay is based on the established monocye derived denticrill cell (MoDC) assay. For this purpose, CD1a+/CD14+ cells were used as antigen-presenting cells. After addition of the photosensitizer and exposure to UV radiation, the release of the pro-inflammatory cytokines TNFα and IL-6 was measured as a measure of cell activation. Results obtained from the in vitro assay were consistent with previously described photosensitizing potentials for all chemicals tested. Thus, this assay allows the evaluation of the photosensitizing potential of substances. Moreover the assessment of their allergic, toxic and toxic potential in this single assay is an additional benefit. To improve the method, currently additional substances are tested at present. The method presented here provides a promising assay for assessing photoallergic and in addition phototoxic potential of relevant substances.

92 ASSESSMENT OF A WHOLE SMOKING IN VITRO EXPOSURE SYSTEM.


We have developed a novel chamber for exposing in vitro air-liquid interface (ALI) cultures on Transwell® inserts to mainstream whole cigarette smoke (Patent Publication: WO 03/100417A1). Endpoints studied include cell viability, oxidative DNA damage, gene expression and cytokine production of putative smoking disease-related mediators. In this exposure system, whole smoke is generated under ISO standard conditions using a Borgwaldt RM20s smoking machine, diluted and delivered to ALI cultures on inserts within the chamber. Dosimetry studies indicate smoking delivery to inserts with 33% being deposited in the chamber and 80-95% of particulates reached the chamber with 33% being deposited in the chamber. Results indicated no significant difference between individual Transwell® cytotoxicity within the chamber, mean cytotoxicity 47.0%±13.5, indicating uniform delivery within the chamber. These results suggest our whole smoke system is a reliable, repeatable and potentially physiologically relevant method for generating and exposing ALI cultures to whole smoke. We propose to further characterize in ALI cultures in terms of specific smoke constituents and use this system for the evaluation of cigarette design modifications intended to potentially reduce the harmful effects of smoke.

93 NNN-INDUCED ACUTE TOXICITY AND CYTOKINE ALTERATIONS IN A LUNG SLICE MODEL.

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Lung cancer accounts for nearly 1/3 of all cancer related deaths in the U.S., with tobacco use being the primary risk factor. Clinical observation, pathologic and epidemiologic studies, and animal studies indicate that chronic inflammation contributes to the development of lung cancer. However, the features of the
inflammatory milieu that promote carcinogenesis have not been clearly elucidated. To study these issues, we developed an in vitro lung slice model that allows rapid analysis of the acute effects of carcinogens. We tested this model using NNK, an established carcinogen in tobacco. Lung epithelial A549 cells were treated for 24 h with 5, 25, 50, 100, 500 or 1000 μM NNK and assessed for acute toxicity by measurement of LDH release. Increased LDH was detected at dosages of 500 and 1000 μM. Based on these data, we tested dosages of 50 and 500 μM in lung slice cultures. Lung from C57BL/6J mice were perfused in situ with cold PBS, instilled with culture medium containing 1% low melting point agarose, and hand-sectioned into 750 μM slices. Slices were cultured in supplemented DMEM on Netwell™ inserts at 37°C with 10% CO2 on a rocking platform in the incubator. After 24 h, lung slices were treated with either 50 or 500 μM NNK or untreated. Slices were harvested 6, 24 or 48 h later for measurement of LDH release into the medium and inflammatory cytokines in tissue homogenates and medium. Cytokine protein was measured using a bead-based mouse 11-plex immunosay (Millipore) on the Bioplex system (Biorad). As compared with controls, LDH release increased at 24 and 48 h after exposure to 500 μM NNK. At 24 h after exposure, cytokines were altered in a dose-dependent manner as follows: GCSF, KC, IP-10 and MCP1 concentrations were decreased in tissue homogenates, and medium. Concentrations of IL-6, GCSF, KC, IP-10 and MCP1 were decreased in the medium. These changes are similar to those reported after in vivo exposure of mice to NNK, thus indicating that short term exposure of lung slice cultures can mirror the acute inflammatory impact of lung carcinogens.

THE EFFECT OF NON-STERoidal ANTi-INFLAMMATORY DRUGS ON THE RESPIRATION OF INTACT HEPG2 CELLS AND ISOLATED MITCHONDRIA.

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed therapeutic drugs used in the treatment of acute and chronic pain. They inhibit cyclooxygenase 1 and cyclooxygenase 2, enzymes involved in prostaglandin biosynthesis. NSAIDs have been associated with hepatotoxicity and gastrointestinal side effects, mitochondrial impairment being implicated as one of the mechanisms for their off-target effects. The recent availability of a 96-well plate platform that measures the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of intact cells, in real-time, using oxygen- and pH sensors, enabled us to study the acute effects of NSAIDs on the respiration of intact HepG2 cells. The OCR of cells is largely a measure of mitochondrial respiration and the ECAR a measure of their glycolytic rate. For comparative purposes, we also examined the effects of NSAIDs on respiration of isolated rat liver mitochondria using an oxygen sensitive phosphorescent probe. The oxicam NSAIDs, Meloxicam, S Sudoxicam, Piroxicam and Tenoxicam, uncoupled respiration both in intact HepG2 cells and isolated mitochondria. Mitochondrial impairment by the oxicams resulted in a decrease in the glycolytic rate of the cells. The salicylic derivative, Diflunisal, was a strong uncoupler of respiration in intact cells and increased their glycolytic rate. In contrast, Acetylsalicylic acid and Sulfasalazine acid caused no impairment of respiration in HepG2 cells. The effects of these 3 NSAIDs were mirrored in isolated mitochondria. Sulindac sulfide, the reactive metabolite of Sulindac, caused inhibition of respiration in intact cells and affected respiration of isolated mitochondria, unlike the parent drug. In summary, many of the NSAIDs that impaired respiration in isolated mitochondria also impaired respiration in intact HepG2 cells.

REAL-TIME CELL-ELECTRONIC SENSING FOR MONITORING THE EFFECTS OF ANTIBACTERIALS AND ANTI-RETROVIRALS IN EUKARYOTIC CELLS.

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Mitochondria have their own DNA (mtDNA) and ribosomes. Thirteen proteins are encoded by mtDNA and made on mitochondrial ribosomes. These proteins are involved in energy production. Antibacterials which target the bacterial ribosome as their primary mode of action can impair protein synthesis within the host's mitochondria since the bacterial ribosome and the host's mitochondrial ribosome share structural similarities. Hence, antibacterials can deplete mitochondrial DNA-encoded proteins. Anti-retrovirals which target HIV reverse transcriptase as their primary mode of action can impair the enzyme responsible for mitochondrial DNA replication as an off-target. This leads to depletion of mtDNA and, hence, to depletion of mtDNA-encoded proteins. Hence, both antibacterials and anti-retrovirals can decrease mtDNA-encoded protein levels which can lead to serious adverse events such as lactacidosis, lipoatrophy, and myelosuppression.

We describe here the use of a Real-Time Cell-Electronic Sensing platform, the xCELLigence system, to monitor the effects of antibacterials and anti-retrovirals on HepG2 cell growth over several days. This platform was a convenient way for monitoring the effects of these drugs in real-time in a non-invasive manner and also enabled us to monitor cell growth after compound removal unlike end-point assays. The xCELLigence system showed that some of the antibacterials and anti-retrovirals had a cytostatic effect on HepG2 cells. Further mechanistic studies were done using a high content screening assay that measures mtDNA-encoded protein levels in cells. Most of the antibacterials and anti-retrovirals which caused a cytostatic effect also caused a decrease in mtDNA-encoded protein levels.
The U.S. EPA ToxCastTM program aims to develop methods for mechanistically-based chemical prioritization using a suite of high throughput, in vitro assays that probe relevant biological pathways, and coupling them with statistical and machine learning methods that produce predictive models (toxicity signatures) for in vivo endpoints from animal studies. Toxicity signatures have been developed and reported for predicting in vivo endpoints such as rat liver proliferative lesions from the ToxCast in vitro data set. To develop these signatures, we used a classification approach in which in vivo toxicity data was dichotomized (each chemical caused or did not cause the disease). The in vitro assay data used in building classification models was characterized by potency (e.g. AC50 values), but quantitative efficacy information (e.g. max fold change or degree of inhibition) was not used. A standard machine learning classification approach was then carried out, with t-test feature selection feeding into stepwise logistic regression. Here we report on the results of adding efficacy information to the modeling effort and inserting variable but controlled amounts of noise to the in vitro and in vivo data in order to understand how measurement variability will affect model stability and performance. As expected, we see a critical level of data noise, above which our ability to make useful predictions of in vivo toxicity is lost. We do see that incorporating both efficacy and potency in vitro data into predictive models results in more robust models of chemical toxicity. This abstract does not necessarily reflect Agency policy.

### References

**ICCVAM RECOMMENDATIONS FOR USING IN VITRO OCULAR TOXICITY TEST METHODS TO IDENTIFY SUBSTANCES NOT LABELED AS IRRITANTS: A BOTTOM-UP APPROACH.**


ICCVAM recently evaluated five in vitro methods as potential replacements for the Draize eye test for identifying potential ocular hazards (i.e., the Bovine Corneal Opacity and Permeability [BCOP], Isolated Chicken Eye [ICE], Isolated Rabbit Eye [IRE], Hen’s Egg Test–Chorioallantoic Membrane [HET-CAM], and Cytosensor Microphysiometer [CM] test methods). None of the methods were considered adequate as complete replacements. However, BCOP and CM were recommended for use in limited circumstances as screening tests in a “bottom-up” approach to identify substances that would not require hazard labeling (NL) for eye irritation. For BCOP, the underprediction of irritants as NL ranged from 0% (0/54) to 49% (0/28) for current EU and GHS classification systems. The lack of hazard labeling for underpredicted substances under the EPA system was considered an unacceptable public health hazard based on the severity, duration, and number of in vivo ocular lesions. Therefore, for use in a bottom-up approach, BCOP is recommended only for current EU and GHS classifications. For CM, the underprediction of irritants as NL ranged from 0% (0/27) to 2% (1/46) for EPA when testing water-soluble surfactants and surfactant-containing formulations. The small number of underpredicted irritants based on the EPA system and the associated in vivo lesions were considered mild enough to permit use of the CM in a bottom-up approach within the limited applicability domain for the EPA, EU, and GHS classification systems. This comprehensive ICCVAM evaluation should facilitate regulatory agency acceptance decisions on the methods and their proposed uses. Industry use of these methods can be expected to significantly reduce animal use for ocular safety testing while continuing to support the protection of human health.

### References

**102 SUPPLEMENTATION OF THE VALIDATION DATABASE FOR THE ISOLATED RABBIT EYE (IRE) ASSAY.**

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Developing robust and updated hazard test methods for detecting adverse effects of chemicals used in workplace and consumer settings relies on validation of alternative toxicological techniques, leading to acceptance by OECD and other sanctioning groups. Significant effort by US, EU, and Japanese scientific/regulatory authorities has been devoted to study of ocular organotypic models leading to validation of 2 irritation tests – BCOP and IRE – which in 2009 were amalgamated into the OECD framework. Data from GSK-sponsored studies showed concordance of 82% for the isolated rabbit eye (IRE) with in vivo irritants and non-irritants. When identifying ocular corrosives, positive predictivity for solid materials was 92% and negative predictivity was 100%. The assay performed less well for liquids with positive predictivity of 43%. These data were used to develop our intelligent test strategy for ocular irritation with the IRE used in combination with the SkinEthic reconstructed human corneal epithelium (SOT 2009, abst. 376). This strategy has
been successfully applied to assess and assign hazard labels to over 50 proprietary materials, primarily solids, in the GSK worker safety test programme without recourse to in vivo testing. Along with review of BCOP and IRE, the IRE assay was considered by ICCVAM and ECVAM. Although IRE results are accepted as a screen for severe ocular irritants by some EU regulators, ICCVAM and ECVAM reviews identified several key areas for IRE database expansion to more positively suggest readiness for formal validation. Chief areas of concern were: 1) lack of data from IRE studies in which multiple endpoints were accrued; 2) relatively high number of irrelevant positive outcomes, especially for liquids; 3) standards for identifying some elements of technique, e.g. use of ultrasonic pachymetry to measure corneal swelling. Work to address these areas using a set of 30 diverse substances from the ICCVAM validation chemical database is underway to aid GSK in-house knowledge building on the IRE.

**103 IN VITRO PREDICTION MODEL AS AN ALTERNATIVE METHOD FOR EYE IRRITATION TEST**


Aims: To evaluate the applicability and predictive capacity of the in vitro prediction model for eye irritation of chemicals. Methods: 26 medicals were assessed in the study, which were tested by the six alternative methods for eye irritation tests, including HET-CAM, CAM-TBS, FLT, IRE, 3T3-NUR cytotoxicity assay and red blood cell (RBC) haemolysis assay, meanwhile the medicals were tested by in vivo Draize test. In vivo tests comparison with in vitro tests was analyzed by SPSS software and P.S. Results: It was shown that ranking correlation and class concordance existed between the six alternative methods and Draize test by applying 26 medicals, the relationship between HET-CAM, CAM-TBS, FLT and MMAS is better, a in vitro predictive model was developed of Y from X, subject to the maximum existence between the six alternative methods and Draize test by applying 26 medicals, the relationship between HET-CAM, CAM-TBS, FLT and MMAS is better. Conclusion: It is suggested that the three in vitro assays, HET-CAM, CAM-TBS and FLT, have good predictive capacity, reproducibility and reliability of the in vitro predictive model are good when compared with Draize test. It is meant that the model is of well application value as a screening test in medical safety evaluation.

**104 MEDIA REFINEMENT FOR A LONG TERM CORNEAL CULTURE MODEL OF EYE IRRITATION AND POST-TREATMENT RECOVERY**

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Long Term Corneal Culture has been proposed as an in vitro alternative to evaluate potential eye irritation and to measure chemical toxicity and post-exposure recovery up to 22 days. Excised porcine corneas were cultured in two types of culture media for up to 21 days. Previous culture procedures resulted in significant corneal swelling, particularly at the endothelial layer, which was reduced when corneas were cultured in base medium Dulbecco’s Modified Eagle Media (DMEM) containing 4% L-Glutamine and 10% Fetal Bovine Serum (FBS). Here we report a media refinement based on a concentration of 5% Dextran used as a deswelling agent evaluated to limit corneal swelling during long term culture. Corneas were cultured either in the base medium (Group A) or in the base medium containing 5% Dextran (Group B) for the entire culture period. Corneas were collected upon arrival (Day 0), Day 1, Day 8, Day 15, and Day 22, were fixed in 10% neutral buffered formalin and hematoxylin & eosin sections were examined for histology analysis. Digital photography was used to test relative cloudiness of the corneas at the specified time points and corneal stromal thickness measurements were taken across the entire cornea and compared among the two groups. The thickness of the corneas increased with the time in culture, regardless of the type of media used (with or without Dextran). However, group A corneas (without Dextran) were significantly thicker than Group B corneas (with Dextran) at all time points. Our data show the ability to successfully culture corneas for 22 days with reduced swelling in DMEM media with 5% Dextran.

**105 A NEW IN VITRO METHOD FOR IDENTIFYING CHEMICAL MEDIATED RESPIRATORY TOXICITY USING MULTIPLE ENDPOINT ANALYSIS AND THE EPI-AIRWAY™ MODEL.**

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Regulatory initiatives such as REACH and Amendment VII of the European Cosmetics Directive have been a major force driving the development and validation of new in vitro methods to replace the use of animals for evaluating the safety of chemical ingredients and finished products. The purpose of this study was to develop a novel in vitro human cell-based approach to evaluate the respiratory toxicity of chemicals. Here we describe the use of the EpiAirway™ 3D human tracheobronchial epithelial model (MxTek, Inc) to identify respiratory toxicity by analysis of cell viability, oxidative stress and expression of genes that regulate apoptosis, chemical metabolism and pro-inflammatory responses. This model was developed using five known respiratory irritants/toxins with well characterized in vivo responses, and doxorubicin, a compound that produces multiple organ toxicity. EpiAirway™ cultures were exposed for 24 and 72 hr at 37°C to concentrations of test compounds that ranged from 1 to 100 μM. Cell viability was monitored by MTT and histology. Oxidative stress was determined by analyzing intracellular GSH levels. Changes in the expression of genes regulating apoptosis (Bax and Bcl2), chemical metabolism (CYP1A1) and the pro-inflammatory response (TNFa, TGFβ, IL-1), IL-6 and IL-8) were determined by qRT-PCR. The results of the five known respiratory irritants/toxins, bleomycin, cadmium chloride, lipopolysaccharide, quartz silica and beryllium sulfate, correlated well with known in vivo responses. Doxorubicin reduced cell viability by 24 hr and intracellular GSH levels by 72 hr, while histological analysis at 24 hr revealed structural damage and cell loss. qRT-PCR results showed doxorubicin substantially induced expression (≥2-fold) of CYP1A1, Bcl2, TNF-α, IL-1 and IL-6 after 24 hr of exposure. These data suggest the EpiAirway™ model combined with multiple endpoint analysis and concentration response data can identify chemical induced respiratory toxicity. In addition, the in vitro markers of toxicity correlate with those observed in vivo.

**106 HISTORICAL DATA ON PERSONAL CARE PRODUCTS OVER FOURTEEN YEARS USING THE CHORIOALLANTOIC MEMBRANE VASCULAR ASSAY (CAMVA) AND BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY (BCOP).**

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The Chorioallantoic Membrane Vascular Assay (CAMVA) and Bovine Corneal Opacity and Permeability Assay (BCOP) are two common assays used to determine ocular irritation for consumer-use products. These assays do not require the use of live animals, provide reliable predictive data, provide results similar to in vivo models and are rapid and inexpensive to conduct. Data from 321 studies performed from 1995 to 2009 (a total of 345 test materials assessed by CAMVA and/or BCOP) were compiled to determine the feasibility of predicting ocular irritation for various formulations. Review of the data from both assays found that hair shampoos, skin cleansers, and hair styling sprays (containing ethanol) were repeatedly predicted to be ocular irritants. In contrast skin lotions/moisturizers were repeatedly predicted not to be ocular irritants. Based on the findings for these product types, future ocular irritation testing (i.e., CAMVA/BCOP) can be nearly eliminated as long as formulations are compared to those previously tested. For example, skin cleanser irritation appears to be solely dependent on surfactant species and level in these formulations.

For other product types (e.g., deodorants, make-up removers, hair styling, body sprays) it was concluded that these products should continue to be tested in CAMVA/BCOP for ocular irritation potential because either significant variability exists in the historical data (non-linear graphs) or the historical sample size is too small to permit definitive conclusions (deodorants, make-up removers, massage oils, facial masks, body sprays, and hair styling products).

**107 ASSESSMENT OF CHEMICAL SKIN SENSITIZING POTENCY BY AN IN VITRO ASSAY BASED ON HUMAN DENDRITIC CELLS.**


Classification of sensitizing chemicals according to their potency would be a major breakthrough since human responses may differ in vigour depending on the concentration but also on the nature of the chemical. Up to now relative potency assessment can only be assessed using animal experiments. In previous research, we developed the in vitro VITOSENS® assay which could classify chemicals as being sensitizing or not, based on expression patterns of a selected gene set in dendritic cells derived from CD34+ progenitor cells in human cord blood (CD34-DC). In this research, we tested whether the in vitro VITOSENS® classification model for skin sensitizers is also able to distinguish between weak, moderate, strong and extremely sensitizing chemicals. To evaluate whether VITOSENS® is able to classify chemicals based on their potency, CD34-DC were exposed to a set of 14 sensitizers (representing different classes of chemicals) for 22 days with reduced swelling in DMEM media with 5% Dextran.

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sulfate, cinnamic aldehyde, 2,4-dinitrobenzenesulfonic acid, tetramethylthiam disulfide, and 2-mercaptobenzothiazole). 2 strong (1,4-dihydroxyquinone and methylidibromo glutaronitrile), and 4 extreme skin sensitizers (bandrowski’s base, damson disulfide, and 2-mercaptobenzothiazole), exposure to the chemicals was performed at concentrations that yielded 80% cell viability and the resulting changes in the expression of 8 biomarker genes were measured using real-time RT-PCR. The induced fold changes of biomarker PBEF1 and the 20% cytotoxic concentration were next included in a statistical model. The Vi-TOSENS® response showed a good correlation with the relative potency values of the sensitizing chemical as determined by animal experiments. Next to an in vitro model for the evaluation of the skin sensitizing potency of chemicals, this approach may contribute to a better understanding of the drivers of skin sensitizing potency.

One characteristic of a chemical allergen is its ability to react with proteins prior to the induction of skin sensitization. The majority of chemical allergens is electrophilic and reacts with nucleophilic amino acids like cysteine or lysine. In the DPRA, test chemicals are incubated for 24 hours with two synthetic peptides containing a cysteine or lysine residue, and the reactions are analyzed by HPLC to monitor the extent of peptide depletion. We have previously demonstrated that reactivity correlates with sensitization potential (Gerberick et al. Tox Sci 2007; 97:417-427). The test set of chemicals has been expanded from 82 to 151 (36 strong, 42 moderate, 35 weak and 38 non-sensitizers), spanning a broad range of reaction mechanisms for modifying proteins. The reactivity data were analyzed against existing Local Lymph Node Assay (LLNA) data using classification tree methodology to rank peptide reactivity as minimal, low, moderate and high. Classification tree methodology was also used to assign test chemicals to groupings based on the United Nations Economic Commission for Europe’s proposed Globally Harmonized System of Classification and Labeling of Chemicals (GHS). This classification system uses LLNA EC3 values to group chemicals into 3 categories: Subcategory 1A (EC3 < 2), Subcategory 1B (EC > 2) and Non-sensitizer. In the cases of misclassification, it is generally an over-classification (for example, 1B chemicals classified as 1A). Of the chemicals that are misclassified, some can be explained by looking at their structures while others were related to incompatibilities with the assay. Aside from its accuracy, the DPRA shows excellent reproducibility as demonstrated by tracking run to run variability of several chemicals.

Chemical sensitization or the probability that a chemical ingredient will cause allergic contact dermatitis in humans is a major concern for pharmaceutical, chemical, personal care, and medical device companies who are developing novel ingredients for their products. An in vitro method capable of replacing the current animal models would be of considerable value. The purpose of this study was to build on our previous work, which centered on the established relationship between reactive chemicals and expression of genes controlled by the antioxidant response/electrophilic response element (ARE/EpRE), by adding additional endpoints and building a larger validation set. The in vitro approach described here encompasses cell viability, direct and indirect chemical reactivity, and ARE/EpRe-mediated gene expression combined with an algorithm that identifies sensitizers, their potency category, and provides an estimated LLNA EC3 value. Human keratinocyte (HaCaT) cells were seeded into 96-well culture plates at an initial density of 12,000 cells/well. The cells were allowed to equilibrate for 48 hr. Six exposure concentrations, ranging from 0.01 to 2500 μM were used for each chemical tested. Following a 24 hr exposure period the cells were harvested for analysis. The expression of 11 genes linked to the ARE/EpRe promoter was evaluated by RT-PCR. 96 chemicals classified as non, weak, moderate, strong or extreme sensitizers were tested in this model. Each chemical was evaluated for viability (MTT assay), GSH binding and gene expression. An algorithm was developed to process the data and provide a toxicity index (TI). The TI was then compared to LLNA EC3 values of reference compounds using exponential regression analysis (R2 = 0.90). To challenge this model, two blinded studies were conducted each with 20 compounds. The sensitivity was determined to be 78% and the specificity was 96%. These data indicate that this in vitro sensitization model can identify and categorize skin sensitizers and may be a viable alternative to animal testing.
permit detailed examination of agents formulated to prevent or mitigate environ-
mental-mediated damage in a format that closes mimics the in vivo environment. The Sta
trast® human skin model is a fully-stratified, multi-layered skin tissue, that contains both epidermal and dermal components and faithfully recapitula-
tes the biological characteristics of human skin. In the present study, we demonstrate
the utility of this skin model to evaluate the skin-damaging effects of two known
environmental insults: cigarette smoke and UV light. Oxidative damage, as meas-
ured by an increase in reactive oxygen species (ROS), was detected after exposure of
StaTrast® human skin tissues to cigarette smoke. Pretreatment of tissues with the antiox-
idant parthenolide-depleted (PD)-Feverfew extract prevented this smoke-induced
response. An increase in ROS formation in UV-irradiated StaTrast® skin tissues was
also mitigated by pretreatment with PD-Feverfew extract, although this protecti
on was not as effective as a full spectrum UVA/UVB sunscreen. UV-mediated cy-
tokine release and cellular DNA damage were also investigated using the Sta
trast® human skin model. IL-1α and IL-1β secretion was shown to increase in
a dose dependent manner upon UV irradiation, however proinflammatory cy-
tokine release was abrogated by pretreatment with sunscreen. Immunohistochemical
detection revealed a substantial increase in the formation of TT dimers in UV-irradiated
tissue skin, but this effect was reduced in tissues pre-
treated with sunscreen. These results demonstrate the ability for the StaTrast®
human skin model to accurately reproduce the in vivo response of human skin to
environmental insults, and to assess agents formulated to prevent or mitigate envi-
ronmental-mediated damage.

**113 METHODS FOR IMPROVING THE INTERPRETATION OF IN VITRO ENDocrine SCREENING DATA: REDUCING FALSE NEGATIVE AND FALSE POSITIVE RESULTS.**

J. P. Pregeren, D. P. Blakeman and J. M. McKim, Jr. CelaTix, Kalamazoo, MI.

A key component to most endocrine in vitro testing strategies is the incorporation
of both estrogen and androgen receptor binding and transactivation assays. The
purpose of this study was to identify potential pitfalls and develop improvements in
data interpretation. Estrogen and androgen activity were determined by using human recombinant estrogen and androgen receptors coupled to fluorescence po-
larization detection and the T47D-KBluc and MDA-kb2 cell lines for measuring transactivation processes. When assessing in vitro endocrine disruption screening data, several sources of false positive and false negative data interpretations should be considered. For instance, competitive receptor binding assays can be subject to receptor denaturation by some test articles which can mimic ligand displacement. Examination of curve fit parameters, such as Hill slope, can assist in the evaluation of competitive versus non-specific displacement. In fluorescence polarization meth-
ods, the incorporation of controls consisting of test chemical and fluorescent label in
the absence of receptor can reveal test chemical signal interference. Transactivation reporter cell models of androgen and estrogen receptor mediated activity can be subject to non-receptor specific signal inhibition, which could be misinterpreted as a false positive antagonism. Solubility and cytotoxicity assess-
ments assist data interpretation. However, inhibition of reporter assay signal can be
more sensitive than the assays used to monitor cell viability. To reduce misinterpre-
tation, additional controls could be incorporated such as conducting incubations in
the presence of excess agonist. Under these conditions the agonist will out-compete
receptor specific antagonism, but not reduce non receptor specific assay signal inhibi-
tion. Edge effect in antagonism models can be partially compensated for by using
a split, top-bottom mirrored plate layout. Confirming data using one or more ad-
ditional assay types can also increase confidence in the final data.

**114 PREDICTIVE CAPACITY OF THE 3T3 NEUTRAL RED UPTAKE ASSAY TO IDENTIFY SUBSTANCES WITH ACUTE ORAL LD₅₀>2000 MG/KG.**

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Recent legislation affecting chemical safety testing such as 7th Amendment of the European Council’s Cosmetic Directive and the new Chemicals Regulation REACH, challenge the toxicity community to develop novel means of deter-
mining the toxicity of chemicals using non-animal methods. It has recently been re-
ported that -87% of the substances in the European Union’s New Chemical
Database (NCD) have acute oral LD₅₀>2000 mg/kg, placing them in a “not classi-
fied” category. Therefore the European Centre for the Validation of Alternative

Methods (ECVAM) organized a trial of the 3T3 Neutral Red Uptake cytotoxicity assay to assess its ability to discriminate between toxic/hazardous (LD₅₀<2000 mg/kg) and not classified (LD₅₀>2,000 mg/kg) substances. Fifty-four coded indus-
trial chemicals and two plant alkaloids were tested in three independent laborato-
ries by slightly different methods. The Health and Safety Laboratory (UK) used a
manual procedure of a previously validated protocol while an automated, robotic
version of the protocol was performed at the Institute for Health and Consumer
Protection (JRC, Italy). The Institute for In Vitro Sciences (USA) assessed a less
cosy, abbreviated version of the protocol targeted at resolving toxicities around
the 2,000 mg/kg cutoff. Results were very similar among the three laboratories and
showed that assay sensitivity and predictive values were up to 100% for identifying
industrial chemicals with LD₅₀>2,000 mg/kg, but both alkaloids were underpre-
dicted. Although specificities were relatively low (<45%), the high prevalence of
non-toxic substances in the NCD indicates that if this in vitro approach was used in
the real-world situation, a large number of new chemicals could be correctly identi-
fied as non-toxic.

**115 THE YEAST RESPONSE TO A BROMINATED FLAME RETARDANT.**


The National Research Council recently envisioned the discovery of “toxicity path-
ways”, the cellular signaling pathways that are induced or perturbed in response to
a toxicant, as a novel approach to chemical toxicity testing. Understanding the toxicity
pathways associated with exposure to flame retardants (FR), a group of chemicals
utilized in products such as furniture and construction materials, is severely limited.
As FR have been implicated in neurological and developmental toxicity, endocrine
disruption, and cancer, it is critical to determine how FR can induce toxicity. It was
hypothesized that a chemical genomics approach using a Saccharomyces cerevisiae
genome deletion library could elucidate the yeast cellular toxicity signaling pathways
associated with exposure to bis(2-ethylhexyl)tetrabromophthalate (TBPH), a
brominated component of the Firemaster 550 (Chemtura Corporation) flame re-
tardant mixture. Preliminary statistical and software analyses have identified genes
coded in the Firemaster 550 genome deletion library that are differentially expres-
sed in response to TBPH, including those involved in DNA repair (XRS2 and RAD52), stress response (YAP1), chromatin modification and remodeling (various components and regulators of the
COMM-pass methyltransferase complex, including SWD1, SWD3, BRE2, and BRE1),
proteasome function (UBP6, UBPI, and BRE9) and signal transduction (CMK1, YCK1, and RAS1), and vacuolar processes (VPS8 and VPS21). Many genes and complexes uncovered during this initial assessment have human orthologs that are
implicated in disease. Future investigations will confirm the role of these genes in
TBPH response, as well as examine possibilities for integrating the data into toxic-
ity pathways that can be utilized to identify genes susceptible to multiple toxicants.

**116 TOWARDS AN AUTOMATED SCORING SYSTEM FOR THE SYRIAN HAMSTER EMBRYO ASSAY.**


The Syrian hamster embryo (SHE) assay is actually the most predictive in vitro alter-
tative to the 2 year rodent bioassay. It is based on the ability of a test agent to in-
duce morphological transformation of SHE cells, which correlates with the poten-
tial to induce carcinogenesis. It possesses the ability to identify genotoxic as well as
non-genotoxic carcinogens. Towards the regulatory acceptance of Cell transforma-
tion assays, a detailed review paper (DRP 31) was issued by the OECD in 2006. The
SHE assay and the Balb/c 3T3 assays were recommended for guideline develop-
ment. A pre-validation exercise lead by ECVAM is near completion on both assays.
Several agents have been tested in the “low pH” version of the SHE assay with a
sensitivity of 86 %, a specificity of 83 % and an 83 % overall concordance with ro-
dent bioassay. Despite its good performances, the SHE assay has suffered a lot of
criticism. This is partly due to the lack of knowledge on the molecular mechanisms
underlying cell transformation. Moreover, the visual scoring of the colonies (which
is tedious and subjective) is far more the greatest weakness of this assay.

In order to ease the scoring of the transformed phenotype and make it more objec-
tive, we are developing an automated scoring system in collaboration with IM-
STAR (a high technology company) through a stepwise approach:

Step 1: Recognition and capture of colonies: Successfully completed.
Step 2&3: Automated scoring of colonies and adaptation of the IMSTAR
STAR (a high technology company) through a stepwise approach:

a) Data collection: Sufficient.“Automatic scanning of SHE colonies by the
Pathfinder™ instrument to SHE plate handling: Successfully completed.

b) Pre-processing: Sufficient.

Step 4: Still to do: Recognition of transformed phenotype. Proof of concept was successful.
The final product will be a system that can handle (unattended) up to 20 plates at once and 60 plates/day. Therefore, developing an automated scoring system for the SHE assay is feasible (In 1996, Ridder GM et al. (Carcinogenesis 18 (1996) 1965-1972) showed a 93% agreement between visual scoring and a computerized image analysis). Doing so may encourage the widespread use of this assay.

**117 MONKEY Gall Bladder epithelial cells: isolation, culture, characterization and application in toxicity evaluation.**

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Hepatobiliary toxicity and cholestasis are often encountered in preclinical toxicology studies during drug development, which may require additional evaluations to understand mechanism or to provide context. Towards this end, methods to culture biliary and gall bladder epithelium from several species have been published but not monkey. Typically, isolation and culture of these epithelial cells are laborious. Yield of primary cells and propagation/retention of epithelial cell characteristics with passage are often identified as key issues. Here, we describe 1) procedures for isolation, culture, and cryopreservation of primary monkey gall bladder epithelial cells, 2) their characterization after multiple passages, and 3) application in toxicity evaluation. Epithelium from monkey gall bladder was mechanically detached and then applied to collagen-coated plates as cell clusters or sheets. Using a defined medium, monolayers were achieved. Cells were harvested using non-enzymatic methods for passage, and at certain passages, cells were cryopreserved. The primary, passaged, cryopreserved cells retained morphological (mucovilli) and biochemical (gamma-glutamyl transpeptidase) markers of gall bladder epithelium. Cells were then plated in 96-well format for use in toxicity evaluation. Alpha-naphthylisothiocyanate (ANIT), as a model biliary toxicant was evaluated in these cells as well as primary monkey hepatocytes and Caco-2 cells. The gall bladder epithelial cells were the most sensitive to ANIT toxicity. Overall, our method yielded successful cultures of gall bladder epithelial cells from monkeys, which can be used in evaluating potential compounds for biliary and gall bladder toxicity.

**118 MICROFLUIDIC HEPA TOCYTE ARRAY FOR LONG TERM DRUG EXPOSURE SCREENING.**

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The ability to assess the impact of synthetic molecules on human liver function is critical to the development of safe and useful pharmaceuticals and commercial chemicals. While current in vitro hepatocyte culture methods are growing in popularity, they do not allow long term dosing experiments. We have developed an innovative microfluidic hepatocyte array (MHA) that maintains isolated human hepatocytes in a physiologically relevant configuration, allowing for sustained viability and metabolic functions for many weeks. The core concept is the engineering of a micro-environment similar to the liver sinusoid. Key characteristics include a high degree of cell-cell contact, an artificial endothelial-like barrier to enhance mass transfer, microfluidic sinusoid channels for drug exposure, and a continuous flow format similar to the micro-vasculature. The MHA is proven to show superior P450 activity compared to Matrigel overlay plates with maintenance of induction/inhibition potential over many weeks. The continuous perfusion format allows collection of flow-through medium for analysis of metabolites during the lifetime of the experiment. Additionally, the system is designed to facilitate cell lysis for mRNA analysis, as well as fluorescent plate reader and microscopy based assays. The MHA is formatted to a 96 well plate, allowing easy integration with automation and standard laboratory equipment. The use of microfluidic culture chambers reduces the number of cells required to collect useful data, allowing more data points from limited cell samples.

**119 ARSENIC REGULATION OF MRNAS IN HUMAN CARCINOCENESIS.**

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Arsenic is a well-studied human carcinogen. The mechanism by which arsenic induces cancer, however, is not well understood. It is known that as a general stress inducer, arsenic can activate kinases leading to the over activation of transcription factors including JNK and NF-kB. These transcription factors are known to regulate the expression of early response genes, and likely to regulate miRNAs. The expression of miRNAs is often altered in cancer and other proliferative disorders. It is highly probable then that miRNAs whose expression are altered by arsenic will play a significant role in carcinogenesis. To test this hypothesis we employed in-depth analysis of the role of arsenic in the transcriptional regulation of the miRNAs, determined targets of these miRNAs which cause carcinogenesis, and studied the overall cellular responses induced by arsenic that are attributable to these miRNAs. Data show that arsenic is capable of inducing the expression of many miRNAs, most remarkably, the miR-190. In silico analysis of possible miR-190 targets indicated that this miRNA may also be involved in tumor formation by targeting multiple proteins including PHLPP, an AKT phosphatase. Kinase activation analysis demonstrated that miR-190 is able to mediate arsenic-induced AKT activation, and that this may occur in a PHLPP dependent manner. Furthermore, overexpression of a miR-190 precursor can enhance the expression of VEGF, a protein downstream of AKT signaling. These data suggest that arsenic is capable of inducing expression of miRNAs which may play a critical role in arsenic-induced carcinogenesis.

**120 EFFECTS OF MIXED TOCOPHEROLS ON ESTROGEN RECEPTOR POSITIVE BREAST CANCER.**

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Breast cancer is the second leading cause of cancer death of women in the United States, despite breakthroughs in therapeutic and dietary regimens that optimally an active area of clinical and epidemiological research. Tocopherols are suggested to reduce an individual’s risk in developing breast cancer because of known anti-oxidative properties. Our laboratory has previously found that mixed tocopherols enriched with gamma tocopherol inhibited n-methyl-n-nitrosourea (NMU)-induced estrogen receptor positive mammary tumorogenesis by disrupting estrogen mediated signaling pathways. The objective of this study was to assess the potential anti-estrogenic action of tocopherols in a well recognized rodent model of mammary carcinogenesis. Female ACI rats were utilized because of the sensitivity to 17beta estradiol to induce mammary tumors. In our short term study, the animals were implanted s.c. with estrogen pellets containing 2.5mg of E2 and treated with 0.3% or 0.5% mixed tocopherols enriched with gamma tocopherol in the diet. Estradiol levels were determined in the serum by enzyme immunoassay. Level of tocopherols and their metabolites of tocopherols were determined in the serum and mammary gland by HPLC analysis. Hematoxylin & Eosin staining shows evidence of mammary hyperplasia in estrogen treated animals. When compared to the control group, the 0.5% mixed tocopherols treated animals had a decreased number of terminal buds. Immunohistochemistry was performed; while there was no significant difference between the control and 0.3% mixed tocopherol group, 0.5% mixed tocopherol treated animals showed increased levels of PPARalpha and a reduction in p-Akt. The preliminary results show that mixed tocopherols enriched with gamma tocopherol may have anti-estrogenic effects.

**121 POTENT MUTAGENICITY OF 3-METHYLINDOLE REQUIRES PULMONARY CYTOCHROME P450-MEDIATED BIOACTIVATION: A COMPARISON TO THE PROTOTYPE CIGARETTE SMOKE MUTAGENS B(A)P AND NNK.**

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3-Methylindole (3MI) is a preferential pneumotoxicant found in cigarette smoke. A number of lung expressed human cytochrome P450 enzymes, including 1A1 and 2F1, catalyze the metabolism of 3MI to reactive intermediates that fragment DNA in a cytochrome P450-dependent manner in primary normal human lung cells in culture, but the mutagenic potential of 3MI has not been well established. In the present study, the mutagenic potential of 3MI was compared to the prototypical cigarette smoke carcinogens benzo[a]pyrene (B(a)P) and 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanone (NNK). 3MI, B(a)P and NNK were incubated with the Salmomella typhimurium strain TA98, which is known to detect the most common subtype of cigarette smoke-induced mutagenicity, frameshift mutations in DNA, with three sources of P450-mediated bioactivation: Aroclor-induced rat liver S9, purified cytochrome P450 2F3, or recombinant cytochrome P450 1A1. B(a)P was mutagenic with as little as 0.1 μM, which increased in a concentration-dependent manner up through 100 μM, in the presence of both S9 and 1A1, but was not mutagenic when 2F3 was used. NNK was mutagenic with a concentration of 1 μM when either S9 or 2F3 were employed, but 1A1 did not bioactivate NNK to mutagenic intermediates at concentrations up to 100 μM. Both 1A1 and 2F3 bioactivated 3MI to highly mutagenic intermediates, but rat liver S9 P450 enzymes did not activate 3MI.
not metabolize 3MI to products that were mutagenic. 100 μM 3MI and B(a)P muta-
tated TA98 to the same extent (approximately 100 revertants per plate) when 1A1 was used. These results indicate that metabolism of 3MI by human lung expressed cytochrome P450 enzymes, but not hepatic P450s, induces comparable mutagenic-
ity to the prototype cigarette smoke mutagens B(a)P and NNK and indicates that 3MI may be a possible pulmonary carcinogen. This work supported by NHLBI Grant HL13645.

122 LUNG CANCER RISKS FROM ASBESTOS EXPOSURE, ASBESTOSIS, AND OTHER FIBROTIC LUNG DISEASES: CASE EXAMPLES OF DISTINGUISHING FACTORS FOR DISEASE CAUSATION ANALYSIS.

R. C. James³, B. D. Kerger⁴ and D. H. Garabrant³. 1HSRL Tallahassee, FL, ²TERRA Inc., Tallahassee, FL and ³University of Michigan, Ann Arbor, MI.

While cigarette smoking continues to be the most prominent and well-defined risk factor for lung cancer in adults over age 50, there is consistent and coherent evi-
dence that fibrotic lung diseases in the presence or absence of smoking can increase lung cancer risks. Cigarette smoking alone can cause fibrotic lung disease (emphy-
sema and peribronchial fibrosis), but nonsmokers also exhibit increased risks of lung cancer associated with a variety of potential underlying causes of lung fibrosis such as occupational pneumoconiosis like asbestosis or silicosis, as well as chronic inflammatory diseases like rapidly progressive interstitial pulmonary fibrosis, rheumatoid arthritis, or repeated bouts of severe interstitial pneumonia. The avail-
able toxicological and epidemiological evidence for asbestosis exposure and lung can-
cer risks on balance supports the premise that lung fibrosis from clinical asbestosis increases lung cancer risk only in more heavily exposed asbestosis workers, and that smoking increases their risk beyond that explained by the fibrotic lung changes alone. In persons with a history of apparently low-level asbestos exposures, distin-
guishing clinical features for evaluating disease causation may include: age; smoking history; ionizing radiation exposure history; presence of clinically-important fib-
rotic changes; presence of asbestos lung fiber counts substantially above back-
ground; clinical evidence confirming asbestosis, silicosis, or other occupational pneumoconiosis; exposure-disease latency and time course of pulmonary fibrosis progression; and family and/or personal medical history that reflects potential metastatic origin of the lung cancer or autoimmune/other diseases leading to fib-
rotic lung changes. These examples illustrate that in the absence of clinical as-
bestosis, underlying diseases unrelated to asbestosis exposure may often provide a more plausible explanation for the onset of lung cancer.

123 NUMERICAL CHROMOSOME ABBERRATIONS IN THE PERIPHERAL LYMPHOCYTES OF WORKERS EXPOSED TO LOW LEVELS OF BENZENE.

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Numerical chromosome aberrations (NCA) are thought to play an important role in carcinogenesis. Benzene, an important industrial chemical and ubiquitous envi-
ronmental pollutant, causes leukemia and is known to induce NCA in peripheral lymphocytes at high doses, but its impact at levels below the U.S. occupational standard of 1 ppm remains uncertain. Thus, we explored the effects of low levels of benzene exposure on NCA in a cross-sectional study of 250 exposed shoe workers and 140 controls conducted in Tianjin, China. Individual benzene and toluene ex-
posure was monitored repeatedly for up to 16 months before phlebotomy. Whole peripheral blood was cultured for 48 hours, and NCA were examined by the classic non-banding assay in 200 metaphases per subject. Our results show that hy-
diploidies, hyperdiploidies, and polyploidies were increased in a dose-dependent manner with benzene exposure (P<0.0001, <0.0001, and <0.05, respectively), but not with toluene. Hydiploidies in workers exposed to <1 ppm benzene (avg. level 0.57 ppm) during the month before phlebotomy was increased compared to controls (P<0.0001). To address the influence of past benzene exposure, we exam-
in ed workers exposed to <1 ppm benzene over the previous year, and a subset with <40 ppm-years lifetime cumulative benzene exposure, and found increased hy-
diploidies in both groups (P<0.01). To exclude the effect of other potential expo-
sures on the association of benzene with hydiploidy, we identified a group of workers exposed to <1 ppm benzene with negligible exposure to other solvents and also found increased hyperdiploidy (P<0.001). In conclusion, exposure to benzene at air levels of less than 1 ppm may cause NCA, induction of which is a potential mechanism underlying the leukemogenesis of benzene, and further elevates con-
cern over the safety of the OSHA PEL of 1 ppm and ACGIH TLV of 0.5 ppm.

124 GASOLINE: UNAPPRECIATED VILLAIN OR UNWARRANTED SUSPECT.


Gasoline generally describes the fuels used in internal combustion engines. It rep-
resents a complex and highly variable mixture of over 500 saturated and unsatu-
rated hydrocarbons, with benzene ranging from the most highly studied com-
ounds due to concerns over its potential health effects. Other suspected or
known carcinogens contained within many forms of gasoline include 1,3 butadi-
en, ethylenebenzene, and methyl-tertiary butyl ether (MTBE). The purpose of this as-
sumption to evaluate and summarize the available data on gasoline exposed co-
horts in the published literature. As a result of significant exposures to chem-
icals or mixtures of chemicals, many of the 70+ studies initially selected for review were felt to be unacceptable. A total of thirty-three studies successfully met our cri-
teria for inclusion. These studies were evaluated for workplace descriptions, cohort demographics, time periods of exposure and follow-up, diagnostic methods, con-
trol population sources, and methods for evaluating exposure. The most frequent endpoints reported were for kidney cancer and one or more subtypes of hematopo-
etic cancers. Similar to kidney and hematopoietic cancers, for other cancer end-
points studied, no underlying pattern or clear association between gasoline and any individual malignancy was apparent across the studies reviewed. The results of our analysis indicate that, 1) gasoline exposures should not be characterized as simply dilute benzene exposures, 2) to date, there is insufficient support to link gasoline exposed populations to any particular type of malignancy, and 3) future studies should regard gasoline exposed populations as having qualitatively different health risks compared to historical studies involving workers exposed to solvents or glues with higher benzene concentrations.

125 INDUCTION OF DNA DAMAGE BY 2, 2-BIS (BROMOMETHYL)-1, 3-PROPANEDIOL IN HUMAN URINARY BLADDER EPITHELIUM CELLS.

W. Kong, R. Kuester, A. Gallegos and L. Sipes. Pharmacology, University of Arizona, Tucson, AZ.

2, 2-Bis (bromomethyl)-1, 3-propanediol (BMP) is a flame retardant found in ure-
thane foams and polyester resins. In a two year dietary study, BMP caused neoplas-
tic lesions at multiple sites including urinary bladder in both rats and mice. The mechanism of its carcinogenic effect is unknown. This in vitro study, using SV-40 immortalized human uroepithelial cells (UROsTa), the DNA binding capacity of BMP and its ability to induce DNA damage were investigated. DNA binding was determined after 24h incubation of UROsTa cells with 14C-BMP (10 μM). The ef-
efficiency of concentration (5-100 μM) and time (1-24h) on single cell DNA strand breaks were assessed by the alkaline comet assay. The subsequent cellular responses to DNA damage were evaluated by the expression of proteins associated with these processes. These biomarkers included DNA damage and growth arrest inducible gene 45 (GADD45) for cell cycle arrest, Poly (ADP-ribose) polymerase-1 (PARP-1) for DNA repair and NF E-2 related factor 2 (Nrf2) for transcriptional activation. The results revealed 5 pmol of 14C-BMP associated with 60 μg DNA (resistant to enzyme treatment and phenol-chloroform and ethanol extraction). Results of the comet assay showed that BMP induced DNA strand breaks within 1-3h. The ex-
tent of DNA damage correlated with concentration and was subjected to rapid re-
pair. Western analysis indicated the level of exposure to BMP caused dose (2.5-50 μM) dependent increases in PARP-1 and Nrf2. In addition, time kinetics studies showed all investigated proteins were up regulated by BMP (10 μM) at 24 h (1.7-2 fold). These results demonstrated that BMP can directly associate with DNA and induce DNA damage in UROsTa cells. The cells initiate a multifaceted cellular re-
response to counteract this damage. How these early genotoxic events related to BMP induced carcinogenesis remains to be established. This research was supported in part by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Science Center Grant Number P3-ES-06694.

126 IN VITRO CYTOTOXICITY AND GENOTOXICITY OF SMOKELESS TOBACCO PRODUCTS.

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With the regulation of tobacco products increasing and, while cigarette smoke in vitro toxicity is extensively studied, it is important to establish methods of ex-
tacting and testing smokeless tobacco products (STPs) which are meaningful and relevant in a regulated environment. The purpose of this study was aimed at, firstly, developing an extraction method for STPs; secondly, testing the different extracts for their mutagenicity, cytotoxicity, and clastogenicity; thirdly, to analyze ten com-
merciably available STP brands by means of the Ames, neutral red uptake, and micronucleus assays. Although there was more than one extraction method explored, the extraction method used for further testing utilized water in a ratio of about one in three STP (W/V) ratio. The samples were treated under two conditions: distilled water alone (neutral pH) and distilled water with sodium nitrite (NaNO2) salt for a final concentration of 7.5mg/ml and adjusted to pH 3 to activate nitrification. The dose range finding experiments demonstrated that the most favorable range for STP samples extracted with distilled water alone were much higher, in the mg/ml range, than that used in cigarette smoke condensate (CSC), in the μg/ml range. The same is true for the STP samples treated in NaNO2 water, although the ranges for the NaNO2 water were not as high as the water alone samples. The in vitro analysis of the ten STP brands showed that, in all assays, the samples extracted with distilled water did not have a dose response behavior. Conversely, the samples extracted with water and treated with activated NaNO2 did have a dose dependent effect. Nonetheless, the mutagenic, cytotoxic, and clastogenic effect observed from the STP samples, were minor when considering the relatively high concentrations tested compared to that of CSC, where extract’s concentrations are about a thousand times smaller.

127 SYSTEMS BIOLOGY OF HUMAN BENZENE EXPOSURE.


Toxicogenomic studies, including genome-wide analyses of susceptibility genes (genomics), gene expression (transcriptomics), protein expression (proteomics), and epigenetic modifications (epigenomics), of human populations exposed to benzene, are crucial to understanding gene-environment interactions, providing the ability to develop biomarkers of exposure, early effect and susceptibility. Comprehensive analysis of these toxicogenomic and epigenomic profiles by bioinformatics, in the context of phenotypic endpoints, comprises systems biology. We have applied a systems biology approach to a molecular epidemiology study of workers exposed to benzene, in order to comprehensively define the mechanisms underlying benzene-induced leukemia. Hematotoxicity, a significant decrease in almost all blood cell types and are multi-faceted. We hypothesize that DIM will be chemotherapeutic in PAH-dependent malignancies. The chemoprotective properties of I3C and its prior supplementation with indole-3-carbinol (I3C) significantly protects against PAH-dependent malignancies. The chemoprotective properties of I3C and its primary acid condensation product, DIM, have been observed in many cancer cell types and are multi-faceted. We hypothesize that DIM will be chemotherapeutic in a human cancer cell population analogous to that observed in our murine transplanted model. To test this we selected CCRF-CEM cells derived from a patient with T-ALL as an in vitro model. Proliferation and viability of cells treated with DIM up to 50 μM were assessed with the ViaCount assay, while propidium iodide intercalation was used to monitor cell cycle progression. Lastly, qPCR arrays were utilized for temporal analysis of mRNA transcripts related to apoptosis. DIM was found to significantly inhibit both CCRF-CEM proliferation (20%) and viability (5%) at concentrations of 10 μM and 25 μM respectively. Increased percentages of apoptosis (38%) and G1/S phase (7%) cells were detectable at 12.5 μM. Analysis of mRNA following treatment with 25 μM DIM found significant modulation of 21 transcripts related to apoptosis. Furthermore, these low concentrations had no significant effect on proliferation or viability of normal human thymus cells. These results suggest that DIM may be a potential treatment option for T-ALL growth at non-toxic and physiologically relevant concentrations. This work was supported by USANA Health Sciences, NIH grant CA90890 and the Linux Pauling Institute.

129 THE ALTERATIONS OF P53 PROTEIN PHOSPHORYLATION AND RIBOSYLATION IN HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO 4-(METHYL-NITRO-SAMINO)-1-(3-PYRIDYL)-1-BUTANONE.

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It has been reported that p53 is stabilized and accumulated in the nucleus through post-translational modifications (PTMs) after DNA damage, such as phosphorylation and poly(ADP-ribosyl)ation in both human bronchial epithelial cells, BEAS-2B. The objectives of this study are to evaluate the changes of p53 phosphorylation and poly(ADP-ribosyl)ation and the protein expression of pol (ADP-ribosyl) polymerase 1 (PARP-1), a highly conserved enzyme which catalyzes poly(ADP-ribosyl)ation, and p21, a downstream target of p53, in BEAS-2B cells after DNA damage induced by NNK. BEAS-2B cells were treated with 150 μM NNK for 72 h. The p53 expression pattern was evaluated by two-dimensional (2D) western blot and the PTMs of p53 were determined by immunoprecipitation and western blot. The protein expression levels of PARP-1 and p21 were also determined by western blot. Our results showed more acidic isoforms of p53 in NNK-treated cells than those in non-treated cells. The phosphorylated p53, particularly at Tyr and Ser sites, and poly(ADP-ribosyl)ated p53 were increased above two fold at 24 h when compared to the vehicle control. Furthermore, PARP-1 protein was up-regulated after NNK treatment, which could enhance p53 poly(ADP-ribosyl)ation. The p21 protein expression level was remarkably increased after NNK treatment, which may result from the p53 activation. These findings suggest that p53 is phosphorylated and poly(ADP-ribosyl)ated in response to DNA damage induced by NNK, and the modified p53 has a tendency to accumulate in the nucleus and cannot be exported by CRM1 from nucleus to cytoplasm, and the activation of p53 may lead to the subsequent induction of p53 downstream targets.

130 MECHANISM AND BIOLOGICAL SIGNIFICANCE OF INFLAMMATION MEDIATORS IN CADMIUM INDUCED ONCOGENESIS IN PROSTATE EPITHELIAL CELLS.

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BACKGROUND: Little is known about the etiology of Prostate cancer, although it ranks as 2nd most cancer in Western men. Chronic inflammation (intrinsic) and carcinogen exposure (extrinsic) are two important causes in tumor development. A causal link between carcinogen exposure, such as exposure to heavy metal Cadmium and chronic inflammation in tumorigenesis remain largely unresolved. We sought to link Cadmium-induced carcinogenesis with pro-inflammatory conditions, using an immortalized prostate epithelial cell line, RWPE-1 as a model. We found a strong link between Cadmium and autocrine activation of pro-inflammatory chemokine, IL-8 that causally promote tumorigenesis. RESULTS: RWPE-1 cells were exposed to 1.0 & 10 μM Cadmium Chloride (CdCl2) respectively. Increased percentages of apoptosis, immune response, and inflammatory response were significantly reduced by treatment of cells with I3C & DIM. Concurrently, we observed a significant effect on proliferation or viability of normal human thymus cells. These results suggest that DIM may be a potential treatment option for T-ALL growth at non-toxic and physiologically relevant concentrations. This work was supported by USANA Health Sciences, NIH grant CA90890 and the Linux Pauling Institute.
of these activities in IL-8 depleted cells strongly implicate IL-8 in prostate carcinogenesis. These findings suggested that Cadmium could trigger a pro-inflammatory-oncogenic response through activation of autocrine IL-8 synthesis & NF-kB activation (NIH R01 AT 003544 & VA MERIT review). (BLL).

**131 DNA ADDUCT FORMATION IN DNA REPAIR DEFICIENT (XPA−/-, P53+/-) MICE FED BENZO[α]PYRENE (BP): A MODEL FOR ESOPHAGEAL CANCER.

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Genetically-altered C57BL/6J mice, deficient in Xeroderma pigmentosum complementation group A (XPA) and the tumor suppressor gene p53, have previously been shown to exhibit a high predisposition to BP-induced tumor development (Hoogervorst et al., Carcinogenesis, 2003). XPA−/-p53+/- mice constitute one of the few models in which esophageal tumors are induced upon feeding BP in the diet, and as such have the potential to become a useful model for investigation of human esophageal cancer thought to be associated with polycyclic aromatic hydrocarbon (PAH) exposures. Here we examined formation of the 10-(deoxyguanosin-N2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPdG) adducts in esophagi, livers and lungs of XPA−/-p53+/- mice fed 100 ppm BP in the diet for 4 weeks. For DNA adduct analysis we used HPLC in conjunction with tandem mass spectrometry (HPLC-MS/MS), which provides values specifically for BPdG. Values are expressed as mean adducts/10^8 nucleotides ± standard error of the mean (SEM). There were no BPdG adducts detectable in any tissue of the unexposed mice. BPdG values for esophagi, 38.7 ± 4.8 adducts/10^8 nucleotides (n=5) in males and 60.4 ± 7.1 adducts/10^8 nucleotides (n=5) in females, were significantly higher than BPdG values for liver and lung (p<0.05 for both). In liver there were 23.2 ± 8.0 BPdG adducts/10^8 nucleotides (n=5) in males and 5.3 ± 0.8 adducts/10^8 nucleotides (n=5) in females. In lung there were 2.3 ± 0.8 BPdG adducts/10^8 nucleotides (n=5) in males and 13.1 ± 3.0 adducts/10^8 nucleotides (n=5) in females. Because substantial numbers of BPdG adducts and tumors are induced in esophagi of XPA−/-p53+/- mice fed BP, this model should be useful for elucidation of mechanistic events underlying the PAH-related carcinogenic process, as well as evaluation of chemoprevention opportunities.

**132 CYTOCHROME P450 2SI INFLUENCES CELLULAR PROLIFERATION IN HUMAN PULMONARY CELLS.

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Cytochrome P450 2Si (CYP2S1) is a drug metabolism enzyme expressed in extra-hepatic tissues throughout development and is elevated in response to hyperproliferative disorders, including psoriasis and cancer. All-trans retinoic acid (RA), an active form of Vitamin A, is a likely candidate for the physiological substrate of CYP2S1. Heterologous expression systems have shown CYP2S1-mediated metabolism of RA at supraphysiological concentrations, however it is unclear whether CYP2S1 metabolism influences retinoic acid metabolism at physiologically relevant concentrations. RA alters gene transcription by binding nuclear hormone receptors and trans activation of downstream target genes. Alterations in retinoic acid and its metabolites ultimately influence cell proliferation. To determine the physiological role of CYP2S1 in pulmonary cells, we performed stable shRNA knock down of CYP2S1 expression in both human lung alveolar (A549) and bronchial epithelial cells (Beau-2B). CYP2S1 mRNA levels were significantly reduced by approximately 70% in both A549 and Beau-2B cells. CYP2S1 protein was reduced by 50% and 80% in A549 and Beau-2B, respectively. Bronchial cells with reduced CYP2S1 expression exhibited elevated rates of proliferation with respect to scrambled controls. Additionally, preliminary proliferation studies have also shown variable responses to retinoids dependent on CYP2S1 expression. Taken together these results suggest that CYP2S1-mediated metabolism alters cellular proliferation by influencing retinoid signaling in human lung cells.

**133 CURCUMIN REGULATES CELL CYCLE PROGRESSION IN A P53-DEPENDENT MANNER IN RESPONSE TO BPDE-INDUCED DAMAGE.

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Chronic exposure to carcinogens such as polycyclic aromatic hydrocarbons (PAHs) is linked to lung cancer. Benzo[a]pyrene diol epoxide (BPDE), a prototypical PAH, is believed to play an important role in lung carcinogenesis by forming bulky adducts on DNA. Although curcumin, a component of turmeric, has been shown to inhibit BPDE-induced damage, its exact mechanism of action remains unclear. p53 plays a key role in signalling a variety of DNA damage, including damage caused by chemical carcinogens. Cyclin D1 was observed, physiologically downregulating the expression of cyclin D1 in response to BPDE-induced damage in lung epithelial cells through the activation of p53. Therefore the aim of this study was to investigate whether curcumin prevents BPDE-induced damage in lung epithelial cells through the activation of p53.

Expression of curcumin pretreatment on cell cycle regulation were determined in A549/LXS5 (p53−/-) and A549/E6 (p53+/-) cells. Curcumin pretreated p53-expressing exhibited enhanced levels of p53, p53 phosphorylated at Ser15 (p53S15), and p21WAF1/CIP in a dose-dependent manner in response to BPDE-induced damage. Phosphorylated retinoblastoma (P-Rb) was down-regulated with curcumin pretreatment. Expression of cyclin A, but not cyclin E, was reduced in response to BPDE damage and S-phase arrest was triggered in p53-expressing BPDE treated cells in a time-dependent manner. These data suggest that curcumin pretreatment increased the accumulation of p53-expressing cells in S-phase by enhancing levels of p53 and p53S15, and decreasing cyclin A. Cell cycle progression was not altered in response to BPDE exposure in p53-deficient cells with or without pretreatment with curcumin. In summary, the present study suggests that curcumin may possess chemopreventive activity against BPDE-induced damage by regulating cell cycle progression via enhanced p53 expression in response to DNA damage. Supported by F31ES016719, R03CA119295 and P30ES014443.

**134 EFFECT OF DIETHYL NITROSAMINE ON CYCLIN D1 AND PCNA EXPRESSION - AN IMMUNOHISTOCHEMICAL PERSPECTIVE.

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Liver tumor development can be divided into three major stages: initiation, promotion and progression. The precise molecular mechanisms involved in the evolution of a normal hepatocyte into a preneoplastic focus and then to hepatocellular carcinoma are not completely understood. Studies have shown that elevated levels of proto-oncogenes like cyclin D1 are seen in the livers of mice and rats treated with carcinogenic or toxic compounds. We hypothesized that cyclin D1 is overexpressed in the livers of rats treated with the genotoxic carcinogen diethyl nitrosamine (DEN). The study was conducted using the Solt-Farber resistant hepatocyte model. Male Fisher 344 rats were subjected to partial hepatectomy and treated with single dose of DEN or vehicle. After two weeks all rats received a selection treatment of three daily doses of 2-acyclicaminolouline (2-AAF) followed by single dose of CCl4 and then three additional treatments of 2-AAE. Two weeks later the animals were euthanized and the liver tissues harvested. The tissues were cryosectioned and stained for gamma-glutamyl transpeptidase (GGT) to identify altered hepatic foci (AHF) and the number and volume of GGT-positive foci were determined. Livers were also paraffin-embedded and serially sectioned for immunohistochemical detection of cyclin D1 and proliferating cell nuclear antigen (PCNA). GGT staining showed formation of distinct AHFs in the DEN treated rats. These are suggested to be the precursors of neoplastic nodules. Foci of hepatocytes showing obvious nuclear staining for cyclin D1 and PCNA were considered positive for carcinogenic events involved in the development of hepatocellular carcinoma. Supported by P42 ES 013661.

**135 THE XRCC1 L360R POINT MUTATION SUPPRESSES PROGRESSION OF CARCINOGEN-INDUCED COLON CANCER.

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Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States and the third most common form of cancer in the world affecting both men and women. A number of epidemiological studies have found an association between diet and cancer and various carcinogens and dietary factors are thought to contribute to CRC. The XRCC1 scaffolding protein interacts with many proteins in the base excision repair and single strand break repair pathways. To further investigate the DNA damage and DNA repair mechanisms associated with carcinogenesis and exogenous sources of DNA damage, we developed an XRCC1 mutant mouse model with a point mutation, L360R, in the highly conserved BRCT1 protein binding domain. This point mutation results in decreased base excision repair function, increased sensitivity to alkylating agents and inability to bind to the DNA damage sensing gene poly (ADP-ribose) polymerase 1 (PARP-1). We investigated the effect of the L360R point mutation by treating mutant mice on the C57BL/6 background, once a week...
for six weeks, with 10 mg/kg azoxymethane (AOM), an alkylating colonotropic carcinogen. All mice were terminated eight months following the last AOM treatment. We show that the mutation had no effect on AOM-induced tumor initiation based on tumor multiplicity (1.2 vs 1.1) and tumor incidence between the two genotypes, 50% for the wild type and 47% for mutants respectively. Surprisingly, the tumor burden was 14.2 mm³ in mutants compared to 22.5 mm³ in wild type (p value 0.05). In contrast, there was 39% gross liver incidence in wild type and 85% gross liver lesions in mutants. We conclude that the XRC1 L360R point mutation increases sensitivity to AOM-induced liver toxicity but suppresses AOM-induced tumor progression in the colon. Further investigation will be of interest to determine the clinical relevance of this point mutation in colon cancer.

Three findings establish that saturated fat causes sustained induction of BaP bio-transformation enzymes and extensive metabolism of this toxicant. As a consequence, the reactive metabolites such as epoxides and quinones generated in colon and liver bind with DNA, form adducts resulting in colon tumors in a subchronic exposure regimen (supported by NIH grants S11ES014156, 1F31ES017391-01, 5T32 HL007735-14 and RO3CA130112-01).

136 DIFFERENTIAL DNA REPAIR IN HEMATOPOIETIC STEM AND PROGENITOR CELLS FOLLOWING GENOTOXIC DAMAGE BY BENZENE METABOLITES. D. Alexander1, M. Zimmerman2, M. T Smith3, E. C. Forsberg1 and M. Camps1. 1Microbiology and Environmental Toxicology, University of California Santa Cruz, Santa Cruz, CA, 2Public Health, University of California Berkeley, Berkeley, CA and 3School of Engineering, University of California Santa Cruz, Santa Cruz, CA.

To gain insight into the mechanisms of benzene-induced leukemia, we quantified DNA damage and repair in highly purified populations of murine hematopoietic stem cells (HSC) and myeloid progenitors (MP) in response to alkylating agents and quinone metabolites of benzene. Acute myeloid leukemia is a paradigm for cancer stem cells with a hierarchy analogous to that seen in normal hematopoiesis. Thus, either HSC or MP are the ultimate targets for the genotoxic insults that give rise to environmentally induced leukemias. Our goal is to determine whether a cell-intrinsic difference in metabolic activation of DNA damaging metabolites by HSC or MP contributes to hematotoxicity and benzene-induced leukemia. Applying a COMET assay, we observed a similar pattern of dose dependent DNA damage for both HSC and MP in response to treatment with direct alkylating agents and ionizing irradiation (IR). Although HSC and MP exhibited equal damage immediately after treatment, HSC showed evidence of DNA repair with lower levels of cell elimination after 24hrs in culture as compared to MP Patterns of DNA damage and repair following hydroxyquinone (HQ) treatment of HSC and MP were analogous to that found with IR and alkyllylation. Our observations of repair in HSC are consistent with findings that long-lived HSC have robust intrinsic cell-type specific survival mechanisms and that their largely quiescent status may allow them to initiate DNA repair using error-prone repair mechanisms, which then render them susceptible to transformation. In contrast, short-lived MP appear molecularly poised to undergo apoptosis and are therefore eliminated in response to DNA damage. DNA damage by benzene metabolites in combination with HSC quiescence may be the key to understanding why HSC are a target for leukemic transformation.

137 POTENTIATING EFFECT OF DIETARY FAT ON BENZO(A)PYRENE (BaP) BIOTRANSFORMATION AND COLON TUMORS IN APCMIN MICE. D. L. Harris1, D. B. Hoof2, L. J. Roberts3 and A. Ramesh1. 1Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN, 2Neuroscience & Pharmacology, Meharry Medical College, Nashville, TN and 3Pharmacology, Vanderbilt University, Nashville, TN.

Our studies thus far have shown formation of colon tumors in APCMin mice subsequent to ingestion of fat containing BaP, an environmental toxicant. These findings have human health relevance in that in US alone, around 60,000 lives/year are lost to colon cancer. Diet and environment have been implicated in the development of sporadic colon tumors. Since biotransformation of toxicants is the prime driving force for carcinogenesis, the objective of this study was to determine how dietary fat potentiates the development of colon tumors through altered BaP biotransformation, using a mouse model. Benzo(a)pyrene was administered to ApcMin mice in 2 doses: saturated- (peanut oil) and unsaturated- (coconut oil) fats at doses of 50 and 100 μg/kg body weight (bw) via oral gavage over a 60-day period. Blood, colon, and liver were collected at the end of exposure period. The expression of BaP biotransformation enzymes (CYP1A1, CYP1B1 and GST) in liver and colon were assayed at the level of mRNA and protein. Tissue samples were analyzed by reverse phase-HPLC for BaP metabolites, and 32P-postlabeling method for BaP-DNA adducts. BaP exposure through dietary fat altered its metabolic fate in a dose-dependent manner, with 100 μg/kg dose group registering an elevated expression of BaP biotransformation enzymes, greater concentration of BaP metabolites, BaP-DNA adducts and more adenomas compared to the 50 μg/kg dose group (p < 0.05). This effect was more pronounced for saturated fat group compared to unsaturated fat group (p < 0.05). These findings establish that saturated fat causes sustained induction of BaP bio-transformation enzymes and extensive metabolism of this toxicant. As a consequence, the reactive metabolites such as epoxides and quinones generated in colon.


Hexavalent Cr(VI) chromium enters the cell as the divalent anion CrO42- through the chloride-phosphate-anionic-intracellular-channel (CIIC1) and interacts with glutathione (GSH). It is established that reduction process of Cr(VI) produces a74 DNA-double-strand-breaks in vitro, via Comet assays as well as the H2AX phosphorylation. While ROS are formed intracellularly from Cr(VI) exposure in the cytoplasm causing p53-independent apoptosis initially, it was followed with a subsequent p53-dependent increase of apoptosis. The persistence of unrepaird DNA double-strand-breaks from the exposure to Cr(VI) brings about an increase of p53 phosphorylation and its localization in the cytoplasm leading to apoptosis via the mitochondrial pathway. We also report here that caspase2 is activated from the exposure of cells to Cr(VI) and Cr(V) model compound, but not to exposure to Cr(IV) model compound. Caspase-2 is the initiator-caspase in the nucleus, which activates the intrinsic-mitochondrial-apoptotic pathway with Bax/p53/PUMA from exposure to genotoxic agents. The implication the differential responses of caspase-2 activation to Cr(VI) and its reductive intermediates Cr(V) and Cr(IV) on the mode of action of Cr(VI) induced apoptosis, cell arrest and carcinogenesis is discussed. Disclaimer: The opinions and conclusions in this paper are those of the authors and do not necessarily reflect their affiliated institutions.

139 PROFILING THE MODE OF ACTION FOR LIVER TUMORS IN B6C3F1 MICE EXPOSED TO METHYL ISOBUTYL KETONE (MIBK). D. Getrie1, N. A. Berdasco1, W. Gulledge2, R. Gingell1 and S. Green1. 1The Dow Chemical Company, Midland, MI, 2American Chemistry Council, Arlington, VA, 3Shell Oil Company, Houston, TX and 4Eastman Chemical Company, Kingsport, TN.

MIBK is used primarily as a solvent in the production of paints, pesticide formulations, adhesives, wax/oil separation, leather finishing, textile coating, and specialty surfactants for inks. Chronic exposure to high levels of methyl isobutyl ketone (MIBK) to B6C3F1 mice in a recent NTP study resulted in a significant induction of hepatic adenomas, but not carcinomas. In order to examine a possible mode of action of MIBK, liver-specific clinical chemistry, histopathology, gene expression, cytochrome P450 enzymatic activity, and hepatocellular proliferation were measured in male B6C3F1 mice exposed to 1800 ppm (NTP high dose) of MIBK via inhalation for 7 days. No treatment-related effects were observed for clinical signs, body weights, liver weights, or clinical chemistry measurements in MIBK exposed animals. Treatment-related histopathologic changes in the liver consisted of very slight hepatocyte hypertrophy (enlargement) with increased cytoplastic eosinophilia in the centrilobular/medzonal regions of the hepatic lobule. These changes were consistent with possible increased smooth endoplasmatic reticulum and induction of cytochrome P450 enzymes. Significant transcript induction was observed for Cyp2b10 (constitutive androstane receptor (CAR) associated gene), however no changes were observed in Cyp1a4 or Cyp3a11, and a slight decrease in expression was seen for Cyp4a10 (peroxisome proliferator-activated receptor alpha [PPARalpha] associated gene). A significant induction of Cyp2b2 enzyme activity (PROD) and hepatocellular proliferation were also observed. Aryl hydrocarbon receptor (AhR) related enzyme activity (EROD) was not altered in this study. The responses observed in this study support a CAR-mediated mode of action for the observed liver tumors, which is of little to no relevance to humans.

140 CANNABINOIDS DECREASE CANCER CELL GROWTH AND INHIBIT SP TRANSCRIPTION FACTORS. S. Sreevalsan1, S. Saei2 and Ann E. Kaminsky1. 1Veterinary Physiology and Pharmacology, Texas A & M University, College Station, TX, 2Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, Texas A & M Health Science Center, Houston, TX and 3Center for Integrative Toxicology, Michigan State University, East Lansing, MI.

In addition to their psychotropic effects cannabinoids (CB) and cannabimimetics are effective anti-cancer agents and inhibit growth of multiple cancer cells and tumors. However, the mechanisms of their antineoplastic activity remain unclear. In
this study we examined the effects of two agents that affect both CB1 and CB2 receptors, namely WIN 55,212-2 (WIN), an annalkylindole derivative, which mimics the effects of conventional cannabinoids, and cannabinoids (CBD), a major constituent in the plant cannabis sativa. WIN and CBD inhibited colon (SW480 and RKO), pancreatic (L3.6plp) and prostate (LNCaP and PC3) cancer cell proliferation and growth inhibition IC50 values for 24 hr were in the range of 5.54-5.83 μM for WIN and 9.01-18.54 μM for CBD in these cell lines.

Treatment of colon and prostate cancer cell lines for 24 hr with 5-7.5 μM concentrations of WIN also decreased specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 that are normally overexpressed in cancer cells and tumors. This was accompanied by decreased levels of Sp-dependent genes associated with cancer cell growth, survival (survivi) and angiogenesis (VEGF). Colon (SW480), pancreatic (L3.6plp) and prostate (PC3 and LNCaP) cancer cell proliferation was also inhibited after treatment with 10-15 μM CBD for 24 hr. CBD-dependent effects on Sp and Sp-dependent genes and inhibition of cell proliferation were observed at similar concentrations of CBD. However, when cells were cotreated with WIN or CBD plus specific CB1 and CB2 receptor antagonists downregulation of Sp transcription factors was not reversed. Moreover, treatment with thiol antioxidants did not block the effects of CB-dependent regulation of Sp1, Sp3 or Sp4. The mechanisms of CB-Sp transcription factor interactions are currently being investigated.

### 141 THE URINARY BLADDER CARCINOPROXUR DOES NOT INDUCE GENOTOXIC EFFECTS IN THE URINARY BLADDER OF WISTAR MALE RATS.

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Propoxur (PPX) is a carbamate insecticide which induced bladder cancer in Wistar rats when fed at 5000 ppm in Altromin 1321 diet (1321). PPX was studied for several key events related to carcinogenicity in the urinary bladders (UBs) of Wistar rats administered the compound for 28 days at 8000 ppm in Prokini Kiba SL 3883 diet (3883), which is similar to the 1321 diet. o-Anisidine HCl (AH) was used as a genotoxic UB carcinogen comparator, and trisodium nitroacetatrate (NTA) as an epigenetic UB carcinogen comparator. For the untreated control and 3 test substance groups (PPX, AH, NTA), 4 additional groups were fed 2% ammonium chloride (AC) in the diet, to acidify the urine since 1321 is reported to increase urinary pH. AC did acidify the urine, as expected, although the 3883 diet itself did not increase pH levels above 8. In the NTA groups, post-embedding assay (NPL), AH produced DNA adducts in the UB urethelium (UBU), whereas PPX and NTA did not. In the alkaline comet assay, AH produced DNA strand breaks (DSBs), whereas PPX and NTA did not. Assessment of UBU cell proliferation as measured by immunohistochemistry of proliferating cell nuclear antigen, revealed that NTA and NTA plus AC increased the replicating fraction (RF). Also AH plus AC increased the RF of UBU, whereas PPX groups were not significantly different from controls. Thus, the results reveal no evidence for DNA binding or DSBs in the UBU by PPX, while confirming UBU DNA damage by AH and showing that NTA does not damage DNA. Also there was no evidence for stimulation or inhibition of DNA synthesis in the UBU by PPX.

### 142 OXIDATIVE DNA DAMAGE AND HUMAN ESOPHAGEAL CANCER RISK IN HUAIAN, CHINA.

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Oxidative DNA damage plays important role in carcinogenesis and 8-hydroxy-2'-deoxyguanosine (8-OHdG), the most common oxidative DNA damage biomarker, has been shown to associate with exposure to many environmental carcinogens and formation of several cancer sites. However, its contributing role to human esophageal cancer remains unclear. To investigate its potential role in esophageal carcinogenesis, we conducted a population-based case-control study, with 188 esophagous squamous cell carcinoma (ESCC) cases and 524 age-, gender-, and residency-matched healthy controls recruited from Huaian, China, a high risk area of ESCC. Levels of 8-OHdG in morning urine was determined by solid-phase extraction coupled with HPLC-electrochemical detection and adjusted by urinary creatinine (mean±SD; range: 1.28-3210.23 μg/mg creatinine), with a median level of 22.04 μg/mg creatinine. The averaged urinary 8-OHdG level in the control group was 23.30 ± 47.32 μg/mg creatinine (range: 0.71 - 817.22) with median level of 13.71 μg/mg creatinine. Levels of 8-OHdG in cases were significantly higher than those in controls (p<0.0001) and the difference remained significant (p<0.0001) after adjustment by age and gender. A marginal significant difference was observed in comparison of distribution of hOGG1 genotypes in cases and controls (p=0.0664) with wild type (Ser/Ser) 38 (20.88%) in cases vs. 70 (14.46%) in controls, heterozygote (Ser/Cys) 79 (43.41%) in cases vs. 251 (51.86%) in controls, and mutant type (Cys/Cys) 65 (35.71%) in cases vs. 163 (33.68%) in controls. These results support the role of oxidative DNA damage in the development to esophageal cancer in this high-risk population.

### 143 DISTRIBUTION AND MOLECULAR DOSE OF INHALATION-DERIVED AND ENDOGENOUS FORMALDEHYDE DNA ADDUCTS SUPPORT CAUSATION OF NASAL CARCINOMA, BUT NOT LEUKEMIA.

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Formaldehyde is classified as a known human and animal carcinogen, causing nasopharyngeal cancer. Additionally, limited evidence for leukemia in humans is available; however, this is inconsistent across studies. Both genotoxicity and cytotoxicity are key events in formaldehyde-induced tumors, but no mechanistic data exist for leukemia. In this study, highly sensitive LC-MS/MS-SRM methods were developed and [13CD2]-formaldehyde exposures utilized, allowing differentiation of DNA adducts and DNA-DNA cross-links originating from endogenous and inhalation-derived formaldehyde exposure. The results show that exogenous formaldehyde induced N-α-hydroxyethyl-N-glycine monoadducts and δ-δ cross-links in DNA from rat nasal mucosa, but did not form [13CD2]-adducts in distant tissues. Furthermore, no N′-HO-CD2-N′-dA adducts were detected in nasal DNA, but high amounts of endogenous formaldehyde dG and dA monoadducts were present in each tissues examined. The number of endogenous δ-δ, N′-δ-dG adducts in 1 day and 5 day nasal DNA samples from rats exposed to 10 ppm [13CD2]-formaldehyde was 1.28±0.49 and 2.43±0.78 adducts/10 7 dG, respectively, while 2.63±0.73 and 2.84±1.13 N′-δ-HOC-H2-dG adducts/10 7 dG and 3.95±0.26 and 3.61±0.95 N′-δ-HOC-H2-dA adducts/10 7 dA were present. No N′-HO-Cδ-dG adducts were detected in lung, liver, spleen, bone marrow or thymus, despite analyzing 5 times more DNA than for nasal epithelium, while endogenous dG and dA adducts were present in amounts similar to nasal DNA. This study provides strong evidence supporting a genotoxic and cytotoxic mode of action for nasal squamous cell carcinoma induction by formaldehyde, but does not support the biological plausibility for the causation of leukemia.

### 144 RESVERATROL-MEDIATED CHEMOPREVENTION OF TCDD-INDUCED SKIN CARCINOGENESIS.

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TCDD, an environmental pollutant causes immunotoxicity, thymic atrophy, and promotes cancer development in humans and animals. TCDD mediates its toxic effects primarily through activation of aryl hydrocarbon receptor (AhR). Resveratrol (RES; trans-3,5,4'-trihydroxystilbene), found in plant products, including red grapes, possesses antioxidant, anti-inflammatory, and anti-cancer properties. RES induced apoptosis in EL-4 tumor cells and blocked tumor generation in mice. Interestingly, apoptosis was mediated through activation of aryl hydrocarbon receptor (AhR) and estrogen receptor (ER). We also noted that RES at lower concentrations acted as an AhR antagonist. Therefore, we investigated whether RES can block TCDD-induced development of skin cancer in mice. To this end, we used transgenic mice (Tg.Ac), which are genetically modified and carry a fusion gene consisting of Zeta-globin promoter, β-Ha-ras gene and an SV-40 polyadenylation sequence. We exposed the skin of Tg.Ac mice by painting TCDD (355 ng/kg body weight equivalent to 71 ng TCDD/kg/day) suspended in acetone on alternate days. The mice were treated with vehicle or RES (100 mg/kg body weight) by oral gavage on alternate days. We observed that mice exposed to TCDD and treated with RES developed significantly delayed and a lower incidence of skin cancer when compared to mice that received vehicle. There was significantly reduced expression of CYP1A1 in the skin (the site of TCDD treatment) and lymph nodes of mice that
received RES post-TCDD exposure when compared to mice that received vehicle. Also, gene array analysis revealed that RES significantly reduced the expression of several genes known to be involved in cancer development and upregulated by TCDD. Moreover, RES reversed the immunotoxicity mediated by TCDD. Together, the data from these studies demonstrated that RES, a natural plant product, can protect mice from TCDD-promoted skin cancer. (This work was supported in part by NIH grants R01ES09098, R01AI058300, R01DA016545, P01AT003961).
149 HISTONE MODIFICATION IN ARSENITE-MEDIATED ANTIOXIDANT GENE REGULATION.


Oxidative stress is implicated in various disease states including neurodegeneration, cancer, and even aging; therefore, understanding how antioxidant enzymes are regulated is important in the investigation of the pathogenesis of oxidative stress-related disease. Several antioxidant enzymes are transcriptionally regulated by a conserved enhancer element, the antioxidant responsive element (ARE). This element is found in the 5' flanking region of such antioxidant genes as NADPH quinone oxidoreductase-1 (NQO1), heme oxygenase-1 (HO1), and the iron binding protein ferritin H, which binds excess iron, thus preventing formation of ROS via the Fenton reaction. We hypothesized that the metalloid arsenite, a potent inducer of histone H3 serine 10 phosphorylation (H3S10P), would stimulate ferritin H transcription through activation of ARE and involve H3S10P. We observed that in human keratinocytes arsenite treatment activated transcription of the ferritin H gene through the ARE via increased NF-E2-related factor 2 (Nrf2) nuclear accumulation, which is the primary ARE transcription factor. The antioxidant N-acetyl-L-cysteine (NAC) reduced Nrf2 nuclear accumulation and to varying degrees, reduced arsenite upregulation of ferritin H, NQO1, and HO-1 genes, suggesting that oxidative stress is involved; NAC also blocked arsenite induced H3S3e10P. In ARE ChIP assays, arsenite induced H3S10P, decreased acetylation of H3K9, and increased methylation of H3K9; this is in stark contrast to increased acetylation of H3K9 during ferritin H promoter activation in response to other ARE activators we tested. These results suggest that histone modifications at H3S10 in conjunction with the change in H3K9 status contributes to arsenite-mediated transcriptional activation of the ARE.

150 REACTIVATION OF L1 RETROTRANSPOSON BY BENZO(A)PYRENE INVOLVES EPIGENETIC MECHANISMS.

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Long interspersed nuclear elements (LINEs or L1 elements) are targeted for epigenetic silencing during early embryonic development and remain inactive in most differentiated cells and tissues via methylation of DNA CpG islands present on their promoter. Conversely, both embryonic stem cells and tumor cells show marked DNA hypomethylation and elevated expression of L1 elements. Although L1 methylation is regulated by DNA methyltransferases, little is known about the transcription factors and mechanisms that regulate L1 expression in the course of development, differentiation, and disease. Recent findings in this laboratory have shown that Rb family members play a role in L1 epigenetic regulation, suggesting that L1 activity is mediated by mechanisms beyond DNA methylation. We sought to study the epigenetic changes that occur at the L1 5'UTR element in Hela cells exposed to BaP, an environmental carcinogen and L1 activator. ChIP assays on the L1 5'UTR demonstrated that (i) short-term exposure to BaP increases histone H3 transcriptional activation marks K9- acetyl and K4-trimethyl, (ii) long-term exposure to BaP reduces the recruitment of both DNMT1 and MB2, but not MB2α3 proteins, indicating L1 activation by BaP. Bisulfite analysis and DNMT siRNA knockdown studies showed that long-term, but not short-term exposure to BaP decreases the methylation levels on the L1 promoter at two different BaP-targeted CpG loci. Thus, the initial control of L1 expression upon BaP exposure is epigenetically regulated via histone changes, while the secondary response is mediated by changes in the recruitment of both DNMT1 and MB2 as well as decreased CpG methylation levels. Overall, L1 is subject to complex regulatory mechanisms involving covalent histone and DNA modifications and recruitment of transcription factors to the L1 promoter.

151 BISPHENOL A UNLOCKS THE RAT PDE4D4 PROMOTER VIA EPIGENETICS.

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Bisphenol A (BPA), a mimic of estrogen, is now used in the manufacture of polycarbonate plastics and epoxy resins in a variety of consumer products. The leakage of BPA from these products makes the xenoestrogen found ubiquitously in the environment and in human bodily fluids and tissues. Its estrogenic effects suggest it can reprogram developing human and animal tissues, particularly those sensitive to estrogens, like the prostate. We previously demonstrated that a CpG island in the phosphodiesterase type 4 variant 4 (PDE4D4) promoter was demethylated in rat prostate neonatally exposed to environmental low dose of BPA or estradiol-17β (E2) when compared to ones in oil-treated controls. Here, we investigate the un-derpinning mechanism of BPA- or E2-induced hypomethylation of PDE4D4 promoter coupled with aberrant over-expression of the gene in an immunortalized normal prostate epithelial cell line (NHe-1). Results illustrated notable difference between the epigenetic regulation of PDE4D4 by E2 versus by BPA. While E2 primarily signaled via a nuclear receptor mechanism, BPA induced PDE4D4 transcription and promoter demethylation via multiple pathways. Thus, its action was blocked by ICI182780, Erk1/2 inhibitor, U0126, and DNA methylation substrate, SAM. Furthermore, the impact of BPA was found to associate with the up-regulation of MB2D and down-regulation of DNMT3A. Reversal of promoter demethylation and gene transcription of PDE4D4 occurred if antisense MB2D DNA oligonucleotides or DNMT3A siRNA were applied. Collectively, these findings support our hypothesis that estrogen mimics epigenetically reprogrammed key pro-static growth regulatory genes in a manner different from E2 through the involve-ment of genomic and non-genomic estrogen action and recruitment of DNA methyltransferases and methyl-CpG binding domain.

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152 REACTIVATION OF THE EPIGENETICALLY SILENCED TUMOR SUPPRESSOR GENE - TISSUE FACTOR PATHWAY INHIBITOR-2 (TFPI-2) BY CURCUMIN CAUSES CELL DEATH IN HEPATOCELLULAR CARCINOMA CELLS.

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Hepatocellular carcinoma (HCC) is considered the fifth most common cancer worldwide and the third most fatal, with a rising incidence in the US as the result of an increase in alcoholic liver disease and obesity. Curcumin, a phenolic compound from the rhizome of the plant Curcuma longa has been shown to inhibit growth and induce cell death in various types of cancer cells including HCC. However, the anti-HCC mode of action of curcumin has not yet been elucidated. In HCC, aberrant promoter methylation and histone deacetylation are implicated in the inactivation of tumor suppressor genes which has a significant impact on carcino-genesis. Tissue factor pathway inhibitor-2 (TFPI-2), a Kunitz-type serine pro tease inhibitor, is a tumor suppressor gene that is frequently epigenetically silenced in human HCC and HCC cell lines. Restoration of TFPI-2 expression in tumor tissue has been shown to not only inhibit invasion, tumor growth, metastasis and an giogenesis but also induce apoptosis. Hence, the goal of this study was to examine the ability of curcumin to reverse the epigenetic silencing of TFPI-2 expression in the HCC cell line HepG2. HepG2 cells were treated with demethylating agent 5-Azacytidine (5-Aza), histone deacetylase inhibitor trichostatin A (TSA) and curcumin. Similarly to 5-Aza and TSA, curcumin was able to significantly re-activate TFPI-2 gene expression in a dose dependent (10 to 50 μM) manner. Further, curcumin in combination with either 5-Aza or TSA was able to robustly re-establish TFPI-2 expression. These data strongly suggest that curcumin can have a therapeu-tic role in the treatment of HCC by reversing the epigenetic alterations involving promoter hypermethylation and histone deacetylation and reactivating the expres-sion of the silenced tumor suppressor gene - TFPI-2.

153 HYPOXIA INDUCES TRI-METHYLATED H3 LYSINE 4 BY INHIBITION OF JARID1A DEMETHYLASE.

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Cancer cells experience severe hypoxia, resulting from reduced oxygen supply from blood vessels because of the rapid cell proliferation. Histone H3 Lysine 4 (H3K4) tri-methylation at the promoter region of genes has been linked to transcriptional activation. In the present study, we found that hypoxia (1 % oxygen) increased H3K4 tri-methylation in both normal human bronchial epithelial Beas-2B cells and human lung carcinoma A549 cells. Hypoxia increased H3K4 tri-methylation by inhibiting H3K4 demethylating activity. In support of this, knocking down JARID1A, which is the major demethylase in Beas-2B cells, attenuated the increase of H3K4 tri-methylation induced by hypoxia. However, the mRNA and protein levels of JARID1A were not affected by hypoxia. GeneChip and pathway analysis in JARID1A knockdown Beas-2B cells revealed that JARID1A regulates the expres-sion of hundreds of genes involved in different cellular functions, including tu-
morgenesis. Knocking down of JARID1A increased H3K4 tri-methylation at the promoters of heme oxygenase-1 (HMOX1) and decay accelerating factor (DAF) genes. These results indicate that hypoxia may target JARID1A which in turn increases H3K4 re-methylation at both the global and gene specific levels, leading to the altered programs of gene expression and tumor progression.

154 ROLE OF EPIGENETIC MECHANISMS IN DIFFERENTIAL REGULATION OF THE DIOXIN-INDUCED HUMAN CYP1A1 AND CYP1B1 GENES.

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The Aryl Hydrocarbon Receptor (AHR)/ARNT heterodimer mediates carcinogenesis by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and certain poly-cyclic aromatic hydrocarbons (PAHs) by activating several genes, including CYP1A1 and CYP1B1. Metabolism of PAHs by CYP1A1 and CYP1B1 plays a major role in carcinogenesis mediated by these compounds. Epigenetic mechanisms that are involved in the dioxin-induced differential regulation of the human CYP1A1 and CYP1B1 genes in MCF-7 and HepG2 cancer cell lines were investigated. RNA PolIII, but not AHR recruitment correlated with the dioxin-induced CYP1A1 and CYP1B1 mRNA expression. Using the DNA methyltransferase inhibitor, 5-AzaC, the role of DNA methylation in silencing was studied. Promoter hypermethylation and silencing was silenced in HepG2 cells due to promoter hypermethylation. Four chromatin modifications of nine studied, AcH3K9, AcH3K14, AcH4, and me3H3K4 were analyzed by chromatin immunoprecipitation. Finally the knock down of JARID1A increased H3K4 tri-methylation at the global and gene specific levels, leading to mis-regulation of target genes. Notably, deficits in hippocampal synaptic function and learning deficits were observed in rats treated with PTU. The slope of contrast-amplitude functions was progressively reduced at each successively higher dose. The results suggest that modest reductions in thyroid hormone limited to the perinatal period are sufficient to permanently alter visual system function in adulthood. These findings are consistent with visual contrast sensitivity deficits seen in children with congenital hypothyroidism. Notably, deficits in hippocampal synaptic function and learning deficits were observed at similar dose levels, suggesting that modest developmental hypothyroidism leads to global physiological deficits in the CNS. (Does not reflect U.S. EPA Policy).
The acute toxicity of toluene, a model volatile organic compound (VOC), depends on the concentration (C) and duration (t) of exposure, and guidelines for acute exposures have traditionally used C x t relationships to extrapolate protective and/or effective concentrations across durations of exposure. Recent research suggests an alternative approach for duration adjustment, which uses PBPK model-derived estimates of internal dose as the basis for duration extrapolation. For example, acute behavioral effects observed in rats performing a visual signal detection task (SDT) were better predicted by the concentration-dose product of exposure. These results confirm the importance of internal dose as the appropriate metric for the acute behavioral effects of toluene and support the use of kinetic models to predict acute effects of longer exposures to VOCs. (This abstract does not necessarily reflect EPA policy.)

Severe developmental thyroid hormone (TH) insufficiency results in alterations in brain structure/function and lasting behavioral impairments. Environmental toxicants reduce circulating levels of TH, but the disruption is modest and the dose-response relationships of TH and neurotoxicity not well defined. Neurotrophins are critical for development of neural connections and for synaptic plasticity. BDNF is highly expressed in developing brain and reduced by severe hypothyroidism. Therefore we induced graded levels of developmental hypothyroidism by exposing pregnant rats to propylthiouracil (PTU, 0, 1, 2 or 3 ppm) in the drinking water from gestational day 0 until postnatal day 21 (PN 22). This regimen produces 20–50% reductions in circulating levels of T4 in dams and pups without altering T3 or postnatal body weight. Animals received drug-free water postweaning, and TH levels return to normal by 2 weeks. One female from each litter was sacrificed on PN14 and PN21, and serum TH and BDNF protein expression determined. Cognitive function was assessed in offspring between PN60–PN90 using trace fear conditioning. At PN91–PN127 rats were sacrificed and brain/blood/brain collected for TH and BDNF protein analysis. Context fear conditioning, a hippocampal-mediated function, was impaired in PTU-exposed males at all doses, but only at the highest dose in females. BDNF declined with age, but was 3-fold higher in hippocampus (Hc) and 33% higher in cortex (Ctx) in adult males than in adult females. BDNF was not altered by PTU at PN14 or PN21. Developmental hypothyroidism reduced BDNF in adult Hc and Ctx at 1 and 2 ppm, but not at the highest dose level. This pattern was more prominent in males. These data indicate that: 1) long lasting changes in BDNF expression emerge in adulthood following developmental hypothyroidism; 2) modest degrees of TH insufficiency are accompanied by cognitive impairments; and 3) a greater TH vulnerability may be present in males than in females. (Does not reflect U.S. EPA policy.)

We have reported developmental neurotoxicity (DNT) induced by prenatal valproate (VPA) exposure using rat fetal brain, and evaluated the usefulness of fetal brain observation in a DNT test. Cortical dysgenesis (hypoplasia of cortical plate) and abnormal traveling of the neural fasciculus at the isthmus, and disturbance of the migration of tyrosine hydroxylase (TH)-positive and serotonin neurons at ventral tegmentum area and raphe nuclei were detected on gestational day (GD) 16 and 20 of VPA-treated fetal brain observations. In this study, we followed these fetal morphological brain changes in postnatal day (PD) 11 offspring treated with 800 mg/kg VPA at GD11, and examined the reliable and sensitive endpoints to detect DNT. Cortical dysgenesis observed in fetal brain was noted as the thinner cortex in offspring. Distribution of Parvalbumin-positive cells, a Ca2-binding protein known to coexist with GABA, in the frontal cerebral cortex was observed in scattered manner in the VPA group, suggesting that VPA affected development of GABAergic neurons. In the pons, a conspicuous round structure was observed in postnatal materials by TH and serotonin immunohistochemistry as similar as in fetal brain observation. In the cerebellum, patchy loss of Purkinje cells in folium VI was observed, although it was difficult to detect the effects of VPA on fetal cerebellum. Thus, most of brain morphological changes in offspring treated with prenatal VPA were reflected on the basis of morphological changes in the fetal brain. These results indicate that VPA-induced-DNT observed on the prenatal brain observation can predict the postnatal brain observation. However, cerebellar observation would be suitable at PD11 to strengthen the results of the fetal brain. (This research was supported by a grant for Long-range Research Initiative from the Japan Chemical Industry Association.)

In contrast to dioxin-like polychlorinated biphenyls (PCBs), systematic knowledge is lacking about non-dioxin-like PCB congeners (NDL-PCBs). Former studies of single congeners have mostly used NDL-PCBs which were not highly purified. As a consequence, the outcome of these studies may have been influenced by Ah receptor-active contaminants. Therefore, various toxicological aspects of highly purified NDL-PCBs were examined within the EU-funded program ATHON. Rat dams were orally given six dose levels of PCB180 (0–1000 mg/kg wt., total dose, prenatal) or PCB52 (0–3000 mg/kg, total dose, pre- and postnatal). Since previous studies revealed pronounced effects of NDL-PCBs and PCB mixtures on the dopaminergic system and auditory function, adult offspring were tested for dopamine-dependent behavior (catalepsy) and brainstem auditory evoked potentials (BAEPs). Latencies to movement onset were determined to evaluate catalepsy induced by IP injection with haloperidol. Male offspring exposed to PCB180 exhibited reduced latencies and significant dose-response relations (p<0.05). In contrast, increased latencies were detected in female offspring exposed to PCB52, but a significant dose-response relation was found only in one of the three test situations (p<0.05). BAEPs were elicited by clicks and tone pips. Slight to moderate elevations of BAEP thresholds were detected in PCB180 exposed females, whereas increases were more pronounced after PCB52, in particular, in males. Dose-response relations were significant in the frequency range from 0.5 to 8 kHz (p<0.05). In conclusion, developmental PCB180 led to more pronounced effects on dopamine-dependent behavior, while effects on auditory function were more expressed after PCB52 (Supported by the EU commission, ATHON, contract FOOD-CT-2005-022923).
Recent reports indicate that 6-12 hours of ketamine anesthesia triggers neuronal apoptosis in postnatal day (PND) 7 rats. In vitro, ex vivo and confocal fluorescent imaging studies suggest that the danyl compounds can accumulate within the cytoplasm of the apoptotic cell. High-resolution positron emission tomography (microPET) imaging has been proposed as a minimally invasive method for detecting apoptosis in the rat brain. Compared with the [18F]-labeled annexin V, which binds to externalized phosphatidylserine (PS) on the outer membrane of apoptotic cells, intracellular uptake of the danylhydrazone of p-fluorobenzaldehyde (DFNSH) may lead to improved target to background contrast ratios. In this study, the effect of ketamine on the uptake and retention of [18F]-DFNSH in the rat brain was investigated using microPET imaging. On PND-7, rat pups in the experimental group were exposed to 6 subcutaneous injections of ketamine and control rat pups received 6 injections of saline. On PND-35, [18F]-DFNSH (37 MBq) was injected into the tail vein of rats and microPET images were obtained over 2 hours following the injection. Radiolabeled tracer accumulation in the region of interest (ROI) in the frontal cortex was converted into Standard Uptake Values (SUVs). After the injection, radiotracer was quickly distributed into the brains of both ketamine- and saline-treated rats. Compared with the control group, the uptake of [18F]-DFNSH was significantly increased in the ROI of treated- treated rats. Additionally, the duration for wash-out of the tracer was prolonged in the ketamine-treated animals. This preliminary study demonstrates that microPET imaging is capable of distinguishing differences in retention of [18F]-DFNSH in different brain regions and suggests that this approach may provide a minimally invasive biomarker of neuronal apoptosis. Supported by NCTR/FDA, E7264

**METHYLPHENIDATE: A THREE-YEAR ASSESSMENT ON COMPLEX BRAIN FUNCTION IN JUVENILE RHESUS MONKEYS.**


Methylphenidate (MPH) is a prescribed stimulant for the treatment of attention-deficit hyperactivity disorder (ADHD), and the widespread use of MPH continues to raise concern about its safety. This study examined the ability of juvenile rhesus monkeys to perform complex behavioral tasks contained in the NCTR Operant Test Battery (OTB) during treatment with MPH that produced clinically-relevant blood levels. Monkeys (n=10/group) were treated orally (2x/day – morning and afternoon) with either 0.5 mg/kg vehicle (CON group), 2.5 mg/kg MPH (LD group), or 12.5 mg/kg MPH (HD group). This dosing regimen provided MPH plasma levels near human therapeutic values (LD group) and five- to ten-times human therapeutic values (HD group). OTB testing (50 minutes/day (M-F)) began 30 minutes after the morning dose and required the monkeys to press levers or press-plates to receive food-pellet reinforcers. The OTB includes Progressive Ratio (PR), Conditioned Position Responding (CPR) and Incremental Repeated Acquisition (IRA) tasks, which assess aspects of motivation, color-position discrimination and learning, respectively. Response Rate (RR), Percent Task Completed (PTC), and Accuracy (ACC) serve as performance metrics. With the exception of elevated serum alanine aminotransferase (ALT) in the HD group, there were no remarkable alterations in other health bio-monitoring parameters. However, there was a significant dose response of MPH on behavior. Monkeys in the HD group performed much more poorly than the LD or CON groups. The HD group lagged well behind in task acquisition and had much lower scores for PTC, RR and ACC in all tasks. MPH is a known appetite suppressant, therefore, much of these effects could stem from a decreased motivation to perform, although effects on ACC suggest other mechanisms. Supported in part by the Best Pharmaceuticals for Children Act 2002 and 2007.
The endogenous cannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) play vital roles during nervous system development including regulating axonal guidance and synaptogenesis. The degradation of 2-AG and AEA is mediated by monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively. Both enzymes are highly susceptible to inhibition by organophosphate compounds in vitro, and acute in vivo exposure of adult animals to the agricultural pesticide chlorpyrifos is related to up-regulation of MAGL activity. A set of recommendations to develop alternative methods for developmental neurotoxicity assessment were prepared to facilitate development of alternative in vitro and in vivo test methods.
These results are comparable to that reported for low-level lead exposure. The hallmark of lead exposure in studies involving children and animals is behavioral deficits. Since in animal studies the most common way to study adverse effects of lead on behavior is operant conditioning, we have used this same behavioral technique in the present study. Animal model studies have shown that prenatal exposure to PAHs causes behavioral deficits accompanied by a downregulation of glutamate receptor subunits. This reduction is postulated to be correlated with impairments in neuronal activity in later life. In this study, we hypothesize that prenatal exposure to benzo(a)pyrene results in deficits in behavioral learning. Timed-pregnant Long Evans Hooded rats were orally exposed to 0, 150, 300 or 600 μg/kg of B(a)P by oral gavage. The disposition of B(a)P to fetal brain was quantified as a function of exposure (E14-E17) during peak periods of neurogenesis for cerebral cortex and hippocampus. Beginning on P60, offspring pups were habituated and tested in a reversal-operant behavioral paradigm. The results demonstrate a dose-dependent decrease in the ability to learn the reversal task with associated decreases in correct responses and an enhancement in incorrect responses in the exposure cohort. Taken together, these studies provide mechanistic insights into how in utero polycyclic aromatic hydrocarbon exposure contributes to adverse neurobiological outcomes.

**172** THE DEVELOPMENTAL NEUROTOXICITY OF MICRONAS: NICOTINE-INDUCED DEFECTS IN MOTOR NEURON AXON GUIDANCE AND BEHAVIOR.

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Previous work demonstrated that embryonic exposure to nicotine in zebrafish results in mispatterning of dorsal-ventral motor neuron axon trajectories. We report that developmental exposure (4-48 hours post fertilization, hpf) to 30 μM nicotine results in defects in secondary motor neuron axon pathfinding and changes in embryonic motor output, a behavioral endpoint used as an indicator of motor neuron developmental abnormalities. Antisense oligonucleotide knockdown of the alpha 2 subunit of the neuronal acetylcholine receptor (nAChR) blocks nicotine-induced effects on behavior suggesting that toxicological effects of nicotine exposure are mediated by inappropriate activation of nAChRs. MicroRNAs (miRNAs) are non-coding RNAs that direct post-transcriptional repression of protein coding genes. Cellular machinery necessary for miRNA biogenesis is only expressed in neuronal axes during development supporting the concept that miRNAs regulate protein synthesis during axogenesis. miRNA and miRNA microarray analyses were conducted at 12, 24, 36, and 48 hpf to identify miRNAs and their putative targets that are misexpressed following nicotine exposure. At time points consistent with secondary motor neuron axon migration, there was a significant change in neuronal-specific miRNA expression relative to time-matched controls. Gene ontology analysis revealed that nicotine exposure disrupts the expression of genes involved in neurogenesis. Taken together, these data indicate that nicotine exposure misregulates the expression of miRNAs and target genes that may collectively choreograph axon outgrowth in developing secondary motor neurons and suggest that miRNA signaling pathways are a target of neurotoxic agents during development. This research was supported by NIH R01 ES016513, P30 ES00210, NIEHS T32 ES07060, and an OSU LPI Pilot Grant.

**173** GENETIC SUSCEPTIBILITY TO PCB-INDUCED MOTOR DYSFUNCTION.

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Polychlorinated biphenyls (PCBs) are man-made industrial chemicals banned over 30 years ago due to their toxicity. Because they are difficult to biodegrade, food and water supplies worldwide still contain measurable levels of many PCB congeners. They are known to cause behavior and memory problems, primarily in children exposed during early development. Two genes important in the metabolism of PCBs are the aryl hydrocarbon receptor (AHR) and Cyp1a2. There are two AHR genetic variants in mice: Ahr6 with poor affinity for PCBs and similar pollutants and Ahr with high affinity. Previous studies showed significant learning and memory deficits in Ahr/Gpr1a2(-/-) mice exposed to PCBs during gestation and lactation. To extend those studies, we examined Ahr/Gpr1a2(-/-) mice with the poor affinity receptor. Pregnant Ahr6/Gpr1a2(-/-) mice were treated orally with a PCB mixture similar to what is found in human breast milk and the food supply, while controls were treated with the corn oil vehicle. Mice were treated at gestational day 10 (GD10) and postnatal day 5 (PND5), which is analogous to the second and third trimester of human brain development. Behavioral testing began on postnatal day 60 (PND 60). We used Novel Object Recognition to assess non-spatial learning and memory and Morris Water Maze to assess spatial learning and memory. PCB-treated Ahr6/Gpr1a2(-/-) mice spent less time exploring the novel object compared with control animals, although the results were not statistically significant (p=0.108). There were significant differences in Morris Water Maze testing. PCB-treated Ahr6/Gpr1a2(-/-) had a higher failure rate (p=0.005) and longer latency to find a hidden platform (p=0.05). These data suggest that Gpr1a2 genotype is important in susceptibility to PCB-induced developmental neurotoxicity.

**174** PERINATAL DIOXIN EXPOSURE INDUCES LOW-DOSE SPECIFIC EFFECTS ON LEARNING AND AFFECTIVE FUNCTION IN ADULT MALE MICE.

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In utero and lactational exposure to dioxins is known to result in behavioral alterations in the offspring of laboratory animals. However, it is largely unknown how dioxins affect advanced brain functions, such as learning and affective function. First, in this study, we developed a novel learning behavioral task by using IntelliCage™, a fully-automated apparatus for behavioral analysis in mice. Next, we studied effects of perinatal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on mice learning behavior in adulthood. Pregnant C57BL/6 mice were given orally either TCDD at a dose of 0, 0.6 or 3.0 μg/kg on gestation day 12.5. After the pups reached adulthood, they were imposed learning behavioral task and completed 57 sessions of the task. A group of mice exposed to 0.6 μg TCDD/kg TCDD (TC-0.6) were found to have impairment in learning throughout the sessions, and suppression of the intensive response activity that control mice had at each session. On the other hand, offspring born to dams exposed to 3.0 μg TCDD/kg TCDD (TC-3.0) did not show a distinct profile from control group in both learning and activity levels. To identify the brain regions which are associated with the behavioral abnormalities, we analyzed immunohistochemically the expression of immediate-early gene Arc/Arc3.1 and c-Fos as the indicator of neuronal activity during behavioral testing. As a result, Arc/Arc3.1 and c-Fos positive cells were found to significantly increase in the central nucleus of amygdala but decrease in the anterior cingulate cortex only in TC-0.6 group, but not in control and TC-3.0 groups, suggesting that the low dose dioxin exposure evokes abnormal activation of limbic emotional circuit. In conclusion, this study demonstrates that perinatal exposure to low dose of TCDD impairs learning and affective function later in adulthood, presumably in a low-dose specific manner.

**175** GENETIC SUSCEPTIBILITY TO PCB-INDUCED DEVELOPMENTAL NEUROTOXICITY.

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Polychlorinated biphenyls (PCBs) are man-made industrial chemicals banned over 30 years ago due to their toxicity. Because they are difficult to biodegrade, food and water supplies worldwide still contain measurable levels of many PCB congeners. They are known to cause behavior and memory problems, primarily in children exposed during early development. Two genes important in the metabolism of PCBs are the aryl hydrocarbon receptor (AHR) and Cyp1a2. There are two AHR genetic variants in mice: Ahr6 with poor affinity for PCBs and similar pollutants and Ahr with high affinity. Previous studies showed significant learning and memory deficits in Ahr6/Gpr1a2(-/-) mice exposed to PCBs during gestation and lactation. To extend those studies, we examined Ahr6/Gpr1a2(-/-) mice with the poor affinity receptor. Pregnant Ahr6/Gpr1a2(-/-) mice were treated orally with a PCB mixture similar to what is found in human breast milk and the food supply, while controls were treated with the corn oil vehicle. Mice were treated at gestational day 10 (GD10) and postnatal day 5 (PND5), which is analogous to the second and third trimester of human brain development. Behavioral testing began on postnatal day 60 (PND 60). We used Novel Object Recognition to assess non-spatial learning and memory and Morris Water Maze to assess spatial learning and memory. PCB-treated Ahr6/Gpr1a2(-/-) mice spent less time exploring the novel object compared with control animals, although the results were not statistically significant (p=0.108). There were significant differences in Morris Water Maze testing. PCB-treated Ahr6/Gpr1a2(-/-) had a higher failure rate (p=0.005) and longer latency to find a hidden platform (p=0.05). These data suggest that Gpr1a2 genotype is important in susceptibility to PCB-induced developmental neurotoxicity.
176 NEONATAL LOW DOSE EXPOSURE OF FEMALE MICE TO NICOTINE ALTERS ADULT SUSCEPTIBILITY TO PARAOXON MANIFESTED AS PERSISTENT NEUROBEHAVIORAL DEFECTS AND INCREASED LEVELS OF PROTEIN TAU.


Low dose exposure of neonatal male mice to nicotine has earlier been shown to cause an increased susceptibility of the cholinergic system at adult age. The present study was undertaken to investigate whether neonatal exposure of female mice to low doses of nicotine alters the adult susceptibility to the organophosphorous compound, paraoxon. Neonatal, 10-day-old, female NMRI mice were exposed to nico-
tine-base (33 or 66 mg/kg b.wt.) or saline s.c. twice daily on 5 consecutive days. At two months of age the animals were exposed to paraoxon (0.25 mg/kg b.wt.) or saline s.c. every second day for 7 days (total of 4 injections/mouse). Spontaneous behaviour in a novel home environment was observed in 2- and 4-month-old mice. Brain homogenates were prepared from cerebral cortex and hippocampus and pro-
tein levels of CaMKII, GAP-43, synaptophysin and tau was measured, using slot-
blot technique. Before the first paraoxon injection, the animals were observed for spontaneous behavior. The spontaneous behavior revealed no differences between the treatment groups. Immediately after the first 60 min observation period the animals received the first injection of paraoxon and were observed for acute re-
ponse to paraoxon. Control animals showed no change in activity whereas mice neonatally exposed to nicotine displayed a decreased activity. Two months after ter-
mination of the paraoxon treatment, the animals were again observed for sponta-
neous behavior. Mice neonatally exposed to nicotine and as adults to paraoxon showed a deranged spontaneous behavior, including hyperactivity and lack of ha-
bitation. These mice also had significantly increased levels of tau in the cerebral 
cortex and in hippocampus. The present study indicates that agents known to affect the cholinergic system in neonates and adults can cause cognitive defects and in-
creased levels of the protein tau, a diagnostic marker for Alzheimer’s diseases.

177 NON-COPLANAR PCBs INCREASE SPONTANEOUS SYNCHRONIZED CALCIUM OSCILLATIONS IN PRIMARY HIPPOCAMPAL NEURONS.

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Polychlorinated biphenyls (PCBs) are group of structurally related chemicals with widespread distribution in the environment. Non-coplanar PCB mediated neurotoxicity may be mediated via Ryanodine-receptor mechanisms modulating Ca2+ de-
pendent events. Primary mouse hippocampal neuronal cultures dissociated from hippocampi of P1 mouse were plated at high density and on day 7 in vitro were exposed to PCBs. Spontaneous oscillations of Ca2+ in hippocampal neurons exposed to solvent alone, PCB 95 (2,2′,3,5′,6-pentachlorobiphenyl) or PCB 66 (2,2′,4,4′-tetrachlorobiphenyl), were monitored using the Ca2+ sensitive dye fluo 4 on day 9 in vitro (48hr exposure). Spontaneous Ca2+ oscillations in neurons exposed to PCB 95 increased in a dose dependent manner (30 to 1000 nM), compared to DMSO control. Neuronal cultures treated with 100, 300 and 1000 nM PCB 95 exhibited significantly increased frequency of spontaneous Ca2+ oscillations in the dendrites and the cell soma (3.5 and 15 fold increase from control for 100, 300 and 1000nM PCB 95 respectively). High frequency oscillations were also observed within an indi-
vidual Ca2+ transient in the neurons exposed to PCB 95 (300 and 1000 nM). Coplanar PCB 66 (100nM) and vehicle treated hippocampal neurons did not dif-
fer in the frequency of Ca2+ transients compared to control (lacking DMSO), nor did they show high frequency oscillations. These results indicate that exposure to non-coplanar PCBs that have no dioxin-like activity alter the fidelity of sponta-
neous intracellular Ca2+ events that contribute to the developing hippocampal neu-
ron. Supported by NIEHS R01 E501-4901.

178 DETECTION OF BRDU-INDUCED DEVELOPMENTAL NEUROTOXICITY BY IMMUNOHISTOCHEMICAL OBSERVATION OF PARVALBUMIN.

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Since 5-bromo-2-deoxyuridine (BrdU), a thymidine analog, is incorporated into the DNA as 5-bromouracil during the synthesis phase of the cell cycle, this agent has been used extensively as a useful tool for labeling proliferating cells. However, several lines of evidence have demonstrated that BrdU is genotoxic. Prenatal expo-
sure to BrdU (50 mg/kg, gestation days 9-15) in the rat, is reported to induce beh-
vioral abnormalities, such as locomotor hyperactivity, impaired learning and memory, and lower anxiety in offspring. So far, we have used this model to establish histological observation of the fetal brain in the developmental neurotoxicity (DNT) test. We detected cell death induction in the intermediate zone and abnor-
mal cortical plate in the developing cerebral cortex, which suggested the induction of impaired migration of GABAergic neurons. To confirm this impairment, we ob-
erved the postnatal brain at 11 days of age. Since parvalbumin (PV) is one of Ca-
binding protein known to coexist with GABA in the cerebral cortex, we carried out PV immunohistochemistry. In the control brain, PV-immunoreactive neurons were observed in layer II-III of the developing cerebral cortex. However, abnormal distri-
bution of the PV-immunoreactive neurons was detected in the BrdU-treated brain. In addition the neurites of PV-immunoreactive neurons in the BrdU-treated brain did not extend as far as in the control brain. PV-immunoreactive neurons with ab-
normal neurites were also observed in the amygdala. The present results suggest that abnormal GABAergic neurons may contribute to BrdU-induced abnormal beh-
vaviors, and that PV-immunohistochemistry is a useful method to detect develop-
mental neurotoxicity in the postnatal brain. (Supported by a grant for Long-range Research Initiative from the Japan Chemical Industry Association)
activity as assessed by lever pressing in the spatial acquisition phase of this para-
digm. However, despite this increased activity, B(a)P exposed Cpr offspring exhib-
itied robust deficits in the retention of spatial discrimination tasks with visual cues. Further, significant impairments are revealed upon presentation of the Cpr exposed
offspring to the within session serial reversal task. The results demonstrate that in
uroto exposure to B(a)P causes deficits in both retention and in cognitive flexibility in
offspring mice. The findings, utilizing this mouse model, support the recent epi-
demiological findings of a PAH exposure associated effect on IQ in a minority
ity cohort of 5 year old children. The findings are significant in that they
deficits in learning behavior (in both children and mouse models of in utero PAH
posure) are similar to that which has been observed in human and animal models
sequent to lead exposure.

subsequent to lead exposure.

demethylated to specific nucleotides in cerebellum and brainstem in rats. To our knowledge, no studies have been conducted to determine if the cy-
totoxic effects of 1,2- and 1,4-DNB parallel those of 1,3-DNB in neuronal or glial
cell models. In previous studies, N-acetyl-L-cysteine (NAC), which increases cellu-
ar GSH was shown to protect oligodendrocytes in vitro against exposure to toxic stimuli such as organo-mercury and to protect cells against programmed cell death associated with exposure to inadequate amounts of trophic factors. The purpose of this in vitro study was to determine if pretreatment with NAC would have a
active effect on C6 Glial cells exposed to individual DNB isomers. Cytotoxicity
was determined using MTT reduction for mitochondrial integrity and neutral red
(NR) uptake for lysosomal integrity. C6 Glial cells were pretreated with NAC for 24
and then exposed individually to each DNB isomer (10–250 μM) for up to 6 hours. The protective effect of NAC on C6 Glial cells exposed to DNB isom-
ers was in the following order 1,2-<1,4-<1,3-DNB using the MTT assay. On the
other hand, using the NR assay, an equal protective effect by NAC was observed
when C6 Glial cells were exposed to the DNB isomers. Based on the results ob-
tained with these two assays, MTT was most sensitive in detecting NAC protection
against DNB toxicity. Our results show that NAC pretreatment has a concentra-
tion- and isomer-dependent effect on cell viability. Studies are underway to explore
the mechanism(s) involved in the ability of NAC to protect cells from DNB toxicity
with record to, and also separate from the glutathione detoxification process.

Dinitrobenzene (DNB) refers to a mixture of three isomers: 1,2-, 1,3- and 1,4-
DNB. The DNBs are used as common intermediates in the dye and plastic indus-
tries. 1,2- and 1,4-DNB cause methemoglobinemia in animal models and in hu-
man. In contrast 1,3-DNB is toxic to specific nuclei in cerebellum and brainstem
in rats. To our knowledge, no studies have been conducted to determine if the cy-
totoxic effects of 1,2- and 1,4-DNB parallel those of 1,3-DNB in neuronal or glial
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the mechanism(s) involved in the ability of NAC to protect cells from DNB toxicity
with record to, and also separate from the glutathione detoxification process.

This project was supported in part by SG12 RR080124 from NCRR at UTep and
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Mitochondrial DNA (mtDNA) damage and environmental exposures have been
related with neurodegeneration, but causation is unclear. The current study in-
vestigates the effect of environmental exposures on mtDNA damage as well as
dopaminergic neurons in C. elegans elegans. In the first experiment, adult
germ-line deficient C. elegans (dp1-1 strain) was exposed to aflatoxin B1, paraquat,
cumene hydroperoxide, rotenone, maneb, manganese, and cadmium. Exposure to
aflatoxin B1 (100 μM), paraquat (20 mM), and cadmium (1 mM) resulted in sig-
nificant dose-dependent mtDNA damage (0.27, 0.84, and 0.30 lesion per 10 thou-
sand base pairs, respectively, p<0.05 in all cases). mtDNA damage, on the
other hand, was detected with aflatoxin B1 (0.08 lesion per 10 thousand base pairs).
No detectable mtDNA damage was found with other chemicals (p>0.05).
Exposure to paraquat (20 mM), cumene hydroperoxide (1 mM), and maneb (754
μM) resulted in a significant dose-dependent decrease in the mtDNA : nuDNA
ratio (11%, 19% and 14% decrease as compared to controls, respectively).
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ratio (11%, 19% and 14% decrease as compared to controls, respectively).
To develop effective means for rapid toxicity evaluation of environmental chemicals, the Tox21 partnership among the National Toxicology Program (NTP), NIH Chemical Genomics Center, and National Center for Computational Toxicology (NCCT) at the U.S. EPA are conducting a number of quantitative high-throughput screening (qHTS) studies with thousands of chemicals. The cell viability qHTS data for an initial set of 1,408 NTP compounds screened in 15-point dose response in 13 cell lines are available in PubChem. We previously showed that biological “descriptors” derived from qHTS IC50 values help to predict in vivo toxicity outcomes of the screened agents using Quantitative Structure-Activity Relationship (QSAR) modeling. However, the full power of the dose-response information of qHTS was not explored. To this end, we have selected 400 qHTS tested compounds, for which binary rodent acute toxicity (i.e., toxic or non-toxic) is known. The classification k Nearest Neighbor (kNN) and Random Forest (RF) QSAR methods were applied using either chemical descriptors alone (conventional models) or in combination with the qHTS-derived biological dose-response profile descriptors (hybrid models). We have also developed special noise-eliminating curve fitting procedures that help address irregularities of the dose-response curves for some compounds and assays. Application of our models to an external dataset resulted in a prediction accuracy of 76% for the conventional models and above 80% for the hybrid models. Moreover, restricting the applicability domain of the hybrid kNN models boosted the prediction accuracy to 86% with only 20% reduction in the chemical space coverage, compared to the accuracy increase but at the expense of 40% loss in the space coverage for the conventional models. Our study confirms that combining in vitro dose-response profiles with conventional chemical descriptors could considerably improve the prognostic power of QSAR models used for in vivo toxicity prediction.

![Image 1](https://example.com/image1.png)

![Image 2](https://example.com/image2.png)

![Image 3](https://example.com/image3.png)

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![Image 9](https://example.com/image9.png)

![Image 10](https://example.com/image10.png)

![Image 11](https://example.com/image11.png)

![Image 12](https://example.com/image12.png)

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![Image 38](https://example.com/image38.png)

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![Image 40](https://example.com/image40.png)
for measured physico-chemical parameter like molar extinction coefficient (MEC) absorption peak were also included. In a last step, the derived predictions were compared to data from in vitro phototoxicity assays (mainly 3T3 neutral red assay).

Our results as well as literature data indicate that a staggered approach could be a useful process to estimate the phototoxic potential of a compound. We suggest to start with a combination of selected molecular descriptors (e.g. HOMO/LUMO gap, conjugated double bonds and LogPeff), followed by physico-chemical parameters (e.g. molar extinction coefficient (MEC)) and in vitro phototoxicity assays.

192 USING TOXCAST IN VITRO ASSAYS IN THE HIERARCHICAL QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) MODELING FOR PREDICTING IN VIVO TOXICITY OF CHEMICALS.

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The goal of chemical toxicology research is utilizing short term bioassays and/or robust computational methods to predict in vivo toxicity endpoints for chemicals. The ToxCast program established at the US Environmental Protection Agency (EPA) is addressing this goal by using ca. 100 in vitro assays to create bioactivity profiles for a set of 320 compounds with known in vivo toxicity measured in ca. 80 assays. The analysis of this data requires new computational approaches to link chemical structures, in vivo responses and in vivo toxicity effects. We have employed a novel hierarchical QSAR approach to develop predictive models of three ToxCast in vivo multi-generation rat toxicity endpoints, i.e., kidney and liver pathologies, and animal viability index. This approach relies on the relationships between in vitro and in vivo assay results as follows: First, all chemicals are partitioned into two classes based on whether the results of the in vitro and in vivo assays agree (i.e., the compound is found either active or inactive in both types of assays) or disagree (the compound’s annotations in vitro versus in vivo disagree). Second, classification QSAR models for these two classes are developed using Random Forest and Support Vector Machine methods. The resulting QSAR models are used to assign compounds in an external dataset to one of the in vitro/in vivo correlation classes and then predict the associated in vivo toxicity based on the known in vitro response. All the ToxCast bioassays were then ranked based on the external predictivity of the associated models for each in vivo toxicity endpoint. The prediction accuracy for all models was in the range of 61-73% for all three in vivo endpoints, while that achieved by conventional QSAR models was only 50-65% for the same external set. Our models could be used to guide the future toxicity studies on the EPA-10K compounds by selecting in vitro assays and prioritizing compounds for in vivo toxicity evaluation.
The metabolic process of steroidogenesis exhibits a complex biochemical topology as the activity of various steroidogenic enzymes control cholesterol metabolism to steroid hormone derivatives. We present a stoichiometric reconstruction of the zebrafish (Danio rerio) steroidogenic network and simulate (using uniform reaction constraints) optimal flux distributions through its various pathways towards steroid metabolite production. The reconstruction defined a set of sixty-five enzyme catalyzed reactions and thirty-seven exchange or transport reactions for steroid metabolites. The reconstructed reactions were inclusive of cholesterol and androgen/estrogen metabolism. Biased (no statement of network objective function) and un-biased (statement of objective function) analyses were applied to identify network properties dependent on reaction stoichiometry. Random sampling of flux distributions through the network identified highly correlated reaction sets that corresponded to the catalysis of steroid metabolites of physiological relevance. Subsequently, optimal flux distributions through network pathways were determined for the production of the three steroidogenic metabolites of: 11-deoxycorti- costerone, testosterone and 17β-estradiol. Furthermore, flux variability analyses revealed physiological feasible pathways for the production of the selected steroidogenic metabolites. The stoichiometric dependence of reactions was also confirmed by conducting deletions of reactions necessary for the production of 17β-estradiol. We demonstrate the potential application of constraint-based reconstruction and simulation techniques in enabling the construction of deterministic and predictive biochemical models. This acknowledgement is poignant considering the susceptibility of the steroidogenic network to environmental and anthropogenic stressors.
Current legislation constraints (7th Amendment to the Cosmetics Directive and REACH) and increasing societal concerns for animal welfare have made the industry enter a new phase in its innovation and R&D process. The challenge is to move from a descriptive to a predictive toxicology implying the development and use of non-animal alternatives. Such a challenge is huge, especially in the area of systemic toxicity as developing an alternative requires the prediction of numerous and complex biological processes. The aim of the present work was to evaluate a multiparameter, cell-based in vitro system for predicting rat acute systemic toxicity. For this purpose, a set of 76 non proprietary chemicals pertaining to different chemical categories were tested using the Ceetox panel® and algorithm developed for the estimation of the LD50 value. Predictive performances of the technology for LD50 determinations demonstrated the ability of such an approach to shed light on potential subcellular targets and also to identify which of the categories defined by the Globally Harmonized System (GHS) were correctly predicted. We showed that chemicals falling below the “non-toxic category” (LD50>2000 mg/kg) were identified with a sensitivity and a specificity of 91% and 78%. At this stage, the Ceetox approach could be used, not to predict the extent and nature of all possible in vivo toxic effects, but rather to estimate the risk of failure if a new chemical entity was to be evaluated with conventional long term in vivo studies.

Certain xenobiotics are sequestered into subcellular organelles by different metabolically-driven mechanisms including mitochondrial membrane potential-driven concentration, nuclear concentration via DNA affinity and vascular-ATPase-driven trapping into lysosomes. This study evaluated, for LD50 thresholds ranging from 100 mg/kg to 2000 mg/kg, the ability of Ceetox panel to identify chemicals falling below the “non-toxic category” (LD50>2000 mg/kg) with a sensitivity and specificity ranging from 91% to 78%. The results showed that chemicals falling below the “non-toxic category” were identified with sensitivity and specificity ranging from 91% to 78%. At this stage, the Ceetox approach could be used to estimate the risk of failure if a new chemical entity was to be evaluated with conventional long term in vivo studies.

Cytchrome P450 (CYP) enzymes are involved in the biotransformation of xenobiotics. Thus, tools and methodologies to predict the rates and metabolites of these and other enzymes are valuable to both chemical and pharmaceutical industries, as well as for direct applications in toxicology. Here, we extend and evaluate the rapid methodology of Korzekwa, Jones, and Gillette (J. Am. Chem. Soc. 1990, 112, 7042-7046) to estimate the activation enthalpy (ΔH) of hydrogen abstraction by CYP enzymes, using the p-nitrosophenoxyl radical (PNPO) as a simple surrogate for the CYP active oxygen species. The ΔH is estimated with a linear regression model using the reaction enthalpy and ionization energy (of the substrate radical) as predictor variables, calculated by semiempirical (SE) methods. Furthermore, our calculated activation enthalpies were compared with those calculated by a hybrid density functional theory (DFT) method, B3LYP, using a more realistic iron-oxo-porphine model, and the results revealed limitations of the PNPO radical model. Thus, predictive models developed using SE predictors provide rapid and generally internally-consistent results, but should be interpreted and used cautiously. This work was supported, in part, by NIHES grant R25 ES012909.
structures of the ligand-protein pairs. Similar constraints have been identified for non-linear regression capabilities. To aid other green chemistry users we created a model builder for multiple green chemistry initiatives. The ability to identify and use relevant sets of compounds to form local QSAR models for multiple endpoints can be later withdrawn due to cardiac adverse events underscores the need for predictive quantitative structure-activity relationship (QSAR) models using the commercial software programs MC4PC and MDL-QSAR. The combined results of the QSAR models improved the overall predictive performance by increasing sensitivity through a consensus weight of evidence approach. These QSAR models may be used as screening tools within the FDA to predict PL and provide decision support to reviewers assessing drug safety.

Drug-induced phospholipidosis (PL) is characterized by phospholipid accumulation and formation of lamellar bodies within lysosomes in various tissues. Although the presence of PL during preclinical testing has led to some regulatory concerns in the past, it remains unclear whether PL causes toxicity. The CDER Informatics and Computational Safety Analysis Staff in collaboration with the Phospholipidosis Working Group has previously developed and published a PL database and predictive quantitative structure-activity relationship (QSAR) models using the commercial software programs MC4PC and MDL-QSAR. The consensus results demonstrated good specificity and sensitivity and supported the known correlation between structural features of cationic amphiphilic drugs and PL. Recently, more than 100 additional positive chemicals were added to the previous database of 190 positives and 393 negatives derived from FDA archives and published literature. A rigorous search of FDA's internal documents identified over 200 additional drugs without PL findings, leading to their inclusion as higher confidence negative compounds that replace some of the lower confidence findings included in the previous model. The new, enhanced database contains approximately 50% PL-positive and 50% PL-negative compounds, which facilitated the development of more robust QSAR models using a selection of fragment-based, topological descriptor-based, and scaffold-based software platforms (MC4PC, MDL-QSAR, Leadscope Predictive Data Miner, and BioEpisteme). In addition, physico-chemical properties of PL-inducing drugs were investigated using ADMET Predictor. The combined results of the QSAR models improved the overall predictive performance by increasing sensitivity through a consensus weight of evidence approach. These QSAR models may be used as screening tools within the FDA to predict PL and provide decision support to reviewers assessing drug safety.

Cardiac safety is a leading cause of pharmaceutical compound attrition and withdrawal of FDA-approved drugs from the market. The underlying mechanisms of cardiomyopathy based cardiotoxicity are poorly understood. Stemina Biomarker Discovery is addressing these issues by combining human embryonic stem (hES) cell derived cardiomyocytes and metabolomics to uncover the metabolic signature of cardiomyopathy inducing drugs. Metabolite biomarkers of cardiotoxicity were discovered and utilized to create a predictive model of toxicity amenable to high-throughput in vitro screening of pharmaceutical agents. hES derived cardiomyocytes supplied by California Stem Cell were exposed to a training set consisting of both chemotherapeutics (anthracyclines, taxanes and kinase inhibitors) known to induce cardiomyopathies and non-cardioxic pharmaceutical agents. The cardiotoxicity of drug treatments was first evaluated by cell viability assays. Then the low molecular weight fraction of the media from the cardiomyocyte cell cultures was analyzed using liquid chromatography and high resolution mass spectrometry. Small molecule biomarkers of cardiotoxicity were identified using both univariate and chemometric based multivariate analysis. Features were selected for predictive models using partial least squares discriminant analysis and random forests. A predictive metabolic signature of cardiomyopathy with the ability to differentiate cardiotoxic from non-cardioxic drug treatments in the training set of compounds was discovered and evaluated by unsupervised statistical analysis. As a result of our improved understanding of the cellular metabolic changes in the cardiomyocytes, we are currently developing an in vitro screen capable of predicting cardiomyopathy inducing properties of pharmaceuticals which will, in turn, lead to greater drug safety.

Phospholipidosis (PLD) is an accumulation of excess phospholipids inside lysosomes, resulting in the formation of multilamellar structures within the affected tissues. PLD is considered an adverse side effect of certain drugs known as cationic amphiphilic drugs (CADs). Herein we introduce a novel high-throughput approach for predicting the PLD-inducing potential of drugs.

Cardiac toxicity is a major cause of drug withdrawal, having been implicated in 28% of drug withdrawals in the USA. The fact that many drugs enter the market to be withdrawn seven years later due to cardiac adverse events underscores the need for predictive quantitative structure-activity relationship (QSAR) models using the commercial software programs MC4PC and MDL-QSAR. The combined results of the QSAR models improved the overall predictive performance by increasing sensitivity through a consensus weight of evidence approach. These QSAR models may be used as screening tools within the FDA to predict PL and provide decision support to reviewers assessing drug safety.
To cause PLD, a drug needs to enter the cell, concentrate in lysosomes, leading to drug-anionic phospholipid complexation. Therefore the extent of the drug-anionic phospholipid complexation should correlate with its PLD-inducing potency. These interactions can be studied using surface tension measurements which were performed using a multichannel microtensiometer system. The surface tension of each drug was measured at 12 different concentrations in the presence of a phospholipid (diC8PS) to establish a surface activity profile.

**Exposure to persistent organic pollutants (POPs)** such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), organochlorine pesticides, hexachlorobenzene (HCB), and phenols causes functional deficits in different organ systems. In the U.S. as well as other countries, environmental exposures to some POPs are reported to be associated with metabolic diseases in the human population. Traditional toxicity testing for such health effects is costly and time consuming. Hence, the National Academy of Sciences has recommended a systems approach of pathway and cellular network analysis to link chemical exposures to health outcomes using emerging technologies as alternatives to traditional testing. The cytochrome P450 (CYP450) enzymes system is a pathway that may play a significant role in POPs-induced metabolic diseases since it processes both xenobiotics and a number of important endogenous molecules.

The U.S. EPA ToxCastTM program is using in vitro high-throughput screening (HTS) assays and legacy databases providing in vivo testing results, sufficiently large data sets are now available for evaluation and potential development of new analysis methods. Correlation of in vitro to in vivo results may be the initial goal of these attempts; however, finding signatures representing class relationships between biological-assays and in vivo effects by navigating through the relevant classes in the chemical domain may improve the prospects of discovering such associations. When relating in vitro observations to in vivo effects, in vitro assay data must be interpreted within a context that also considers the metabolic fate of the chemicals, whereas species-specific metabolism is intrinsically reflected in in vivo experiments. In this paper, an informatics approach is applied to a large dataset from the ToxCast™ Phase I project. We expand the chemical classifiers by including metabolic reactivity indicators to help elucidate in vivo to in vitro relationships at both compound and chemical feature levels. The chemical feature hyperspace will be further characterized by P450 isoform activities and physicochemical properties to augment metabolic reactivity classifiers. This work is a collaborative effort involving regulatory agency, industry, software provider, and academic research groups to establish methods to incorporate metabolic knowledge into new in vitro HTS approaches. This abstract has been reviewed by FDA and EPA, but does not necessarily represent policies of either Agency, nor is it an endorsement of products implied.
213 A DETAILED DESCRIPTION OF A FOOD ADDITIVES KNOWLEDGEBASE AND APPLICATION TO TOXICITY ENDPOINTS: DATABASE, ALERTS, AND QSAR MODELS IN THRESHOLD OF TOXICOLOGICAL CONCERN APPROACH.


Over the past 10 years, FDA's Center for Food Safety and Applied Nutrition, Office of Food Additive Safety (OFAS), in collaboration with FDA's Center for Drug Evaluation and Research Informatics and Computational Safety Analysis Staff, has, through various cooperative research and development agreements, acquired access to several quantitative structure-activity relationship (QSAR) programs and chemoinformatics systems. These software programs are being used in the evaluation of new food contact materials, food additives, dietary ingredients, impurities and breakdown products. An important aspect of creating better QSAR models is the identification and incorporation of high quality toxicity data into QSAR training sets. To this end, OFAS has begun to capture its historical data in a structure-searchable format and to incorporate the information into the existing QSAR models. Work has begun to transform information in this database into a new food additives knowledgebase. The food additives knowledgebase consists of the aforementioned database, structural and biological rules, and mode of action driven QSAR models. A peculiarity sequence database, along with the concern factors (defined as exposure divided by concern level) are based on the threshold of toxicological concern (TTC) approach and can be stratified across multiple toxicity endpoints allowing for the pre- and post-market evaluation of food additives. Knowledge derived from this evaluation can be used to eliminate unnecessary toxicity testing or to identify new safety concerns as new exposure and toxicity data are incorporated into the model. The knowledgebase modules are designed to be delivered within a configurable web-based workflow management tool. Demonstration of application of this approach to toxicity endpoints is presented in this paper. This abstract has been reviewed by FDA, but does not necessarily represent Agency policy.

214 WWW.ALDH.ORG, A WEB DATABASE FOR THE CHARACTERIZATION AND REPORTING OF GENE SUPERFAMILIES.

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The World Wide Web has revolutionized and expedited the way investigators gather information for their specific areas of interest. Nucleic Acids Research has reported over 1000 online databases in its Molecular Biology Database Collection. These computer-assisted relational databases and software applications for genomic, transcriptomic, proteomic, and structural analyses allow the researcher with the ability to take vast amounts of information and distill it to develop new assumptions, hypotheses and eventual theories. However, the independent nature of these databases has lead to an intractable number of identification numbers and naming conventions per gene, transcript and peptide often with disregard for sequence integrity, splice variants, and gene sequence. This has occurred because of a host of factors including a) a lack of gene naming conventions, b) numerous accession identification systems per gene transcript/peptide, c) sequence discrepancies due to alternative splicing, single nucleotide polymorphisms, insertions and/or deletions, and, d) a variety of independent sequencing projects. This has inevitably led to many discrepancies in information between and within databases and in the published literature. The ALDH.ORG web database prototype is a web-based software solution developed to address a number of the above issues using a systematic architecture for the curation, characterization and reporting on a gene superfamily to the internet. Using the Aldehyde Dehydrogenase (ALDH) gene superfamily as an example, the database utilizes a curation engine to incorporate specific gene records for this gene superfamily from multiple informational tributaries (i.e. NCBI and EBI sequence databases, UniProt, PubMed, etc.). The purpose of this curation engine prototype is to assemble as a resource, a clear, concise, accurate referenced informational data-base. It combines new and existing biomolecular information for ALDH genes, databases, UniProt, PubMed, etc.). The purpose of this curation engine prototype is to assemble as a resource, a clear, concise, accurate referenced informational data-base. It combines new and existing biomolecular information for ALDH genes, databases, and informatics perspectives, reducing 300 unique chemicals to a smaller number of chemically or biologically informed classes can potentially improve the likelihood of discerning in vitro to in vivo associations. The ToxCast Phase I chemical inventory was characterized into classes and clusters according to various chemical structure-based and biologically supervised descriptors, including quantum mechanical, feature-based, ADME-based, and QSAR-based. These different types of classifiers were chosen to offer alternate hypotheses for partitioning chemical space according to plausible mechanistic drivers. In addition to global comparisons of classifiers across the entire 304 library, we examined how chemicals within specific chemical classes, such as “organophosphates”, will partition differently according to these various classification methods. These approaches are designed to be extendible to the next phases of the ToxCast program, which will expand and enrich the current chemical library. This abstract has been reviewed by EPA, but does not necessarily represent Agency policy.

216 RDX BINDS TO THE CONVULSANT SITE OF THE GABA RECEPTOR AND INCREASES SPONTANEOUS FIRING RATES OF CORTICAL NEURONS IN VITRO.

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RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine, hexogen, Royal Detonation Explosive) is an explosive widely used by the military and has been found in soil and groundwater in and surrounding training ranges, creating potential hazards to the environment and human health. Oral RDX over-exposure results in development of convulsions and epileptiform seizure in quail, mice, rats, monkeys and humans. The mechanism of RDX-induced seizure is unknown. In this work, RDX was screened for affinity against a battery of neurotransmitter receptors and found to bind exclusively to the convulant site on the GABAA receptor. RDX competitively displaced [35S]BPS binding from GABAA receptors with an IC50 of 22 μM, compared to an IC50 of 233 nM for picrotoxin. To assess the functional ramifications of RDX interaction with GABAA receptors, RDX effects were then examined in a well characterized model of cortical network electrophysiology, i.e., primary cortical neurons grown on microelectrode arrays (MEAs). After 14-30 days in vitro, these cultures develop stable, spontaneous network activity in the form of action potentials (APs) and bursts of APs. Acute exposure to RDX caused a concentration-dependent increase in the rate of spontaneous AP firing with an EC50 of 12 μM. RDX also increased the rate and duration of bursts of APs, as well as the % of APs that occurred within a burst. The GABAA receptor antagonist bicuculline caused similar changes in spontaneous activity. These data indicate that inhibition of GABAA receptors and the resultant disruption of spontaneous neuronal network activity may underlie the seizure-inducing activity of RDX. (This abstract does not reflect EPA policy).

217 RELEASE OF CALCIUM FROM INTRACELLULAR STORES IN PC12 CELLS BY HYDROXYLATED METABOLITES OF BDE-47 IS STRUCTURE-DEPENDENT.

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Recent research demonstrated that a hydroxylated metabolite of the environmentally relevant polychlorinated diphenyl ether (PBDE) congener BDE-47 (2',4,4'-tetraBDE) increases neurotransmitter release by releasing Ca2+ from intracellular stores at lower concentrations than its parent congener. Several other hydroxylated metabolites of PBDEs (OH-PBDEs) also were detected in human urine and cord blood. To investigate the neurotoxic potential of environmentally relevant PBDEs and their metabolites, the acute effects of several parent PBDEs (BDE-47, BDE-49, BDE-99, BDE-100, BDE-153) and metabolites of BDE-47 (4'-OH-BDE-47, 4'-OH-BDE-49, 5-OH-BDE-47, 6'-OH-BDE-49 and 6-OH-BDE-47) and its methoxylated analogue 6-MeO-BDE-47 on the intracellular Ca2+ concentration ([Ca2+]i) were measured using the Ca2+-responsive dye Fura-2 in neuroendocrine pheochromocytoma (PC12) cells.
In contrast to the parent PBDEs and 6-MeO-BDE-47, all investigated OH-PBDEs induced CYP release from intracellular stores, although with different lowest-observed-effect-concentrations (LOEcs). The intracellular CYP sources involved in the observed increases in [CYP], were either endoplasmic reticulum (ER) or both ER and mitochondria. When investigating fluctuations in [CYP], a more subtle endpoint, lower LOEcs were observed for 6-OH-BDE-47 and 4'-OH-BDE-49, as well as for BDE-47. The combined data revealed that shielding of the OH-group on both sides with Br-atoms and/or the ether-bond to the other phenyl ring (as in 6'-OH-BDE-49 and 3-OH-BDE-47) lowers the potency of OH-PBDEs. The present findings demonstrate that the hydroxylated metabolites of BDE-47 cause disturbance of the [CYP],. This indicates that bioactivation of PBDEs by oxidative metabolism should be included in human risk assessment of persistent organic pollutants.

PHARMACOKINETIC PROFILES OF PERFLUOROOCTANOIC ACID IN MICE AFTER CHRONIC EXPOSURE.


Perfluorooctanoic acid (PFOA) is a highly persistent, ubiquitous environmental contaminant and a toxicant, which is bioaccumulated in tissues of exposed animals and humans (Sgro et al., 2012). The results of this study, which is the first to assess the pharmacokinetic profile of PFOA in mice after a single oral administration, provide important new information about the metabolism of PFOA in mice.

TOXICOCENOMIC PROFILING OF PERFLUORONONANOIC ACID IN WILD-TYPE AND PPARα-NULL MICE.


Perfluorononanoic acid (PFNA) is a ubiquitous environmental contaminant and a developmental toxicant in laboratory animals. Like other perfluoralkyl acids (PFAAs), PFNA is a potent activator of PPARα and PPARγ and displays greater acute toxicity. In the current study, accumulation of PFNA in serum and liver was evaluated after repeated treatment at various doses. Young adult female CD-1 mice were given PFNA once daily by oral gavage at 0.1, 1, or 10 mg/kg. At the time of sacrifice, plasma and liver samples were collected for PFOA determination by HPLC-MS-MS. Dose-dependent elevations of liver weight were observed in the 1 and 10 mg/kg, groups, but not at 0.1 mg/kg. At 0.1 mg/kg, serum concentrations of PFOA increased linearly with treatment duration, and the concentrations of PFOA in liver appeared to mirror those of serum (ratio concentrations serum:liver 1.1). At 1 mg/kg, serum concentrations of PFOA also increased with treatment duration, but levels of the chemical in liver were about twice as high as those in serum. At 10 mg/kg, PFOA concentrations rose rapidly both in the serum and liver, but appeared to reach steady-state levels after one week of repeated exposure, however, levels of PFOA in liver were about twice of serum levels after 7 weeks (similar to the mg/kg group). The enhanced accumulation of PFNA in the mouse liver correlated well with increases of liver weight (r² = 0.98). These results suggest that serum and liver accumulation of PFNA and elimination kinetics are dependent on exposure doses, likely involving saturation of hepatic and renal transporters at the high doses. This abstract does not necessarily reflect U.S. EPA policy.

EFFECT OF ORGANOCHLORINE COMPOUND EXPOSURE ON ADIPOGENESIS AND ADIPOKINE PRODUCTION IN NIH3T3-L1 CELLS.

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Adipogenesis is a key step in the formation of obesity, which is approaching epidemic proportions in the United States. Recently, exposure to the organochlorine (OC) compounds dichlorodiphenyltrichloroethane (DDE), oxychlordane, and trans-nonachlor has been associated with the prevalence of metabolic syndrome and diabetes. Thus, the current study seeks to determine the effect of these compounds on adipogenesis and adipokine/cytokine production, a key process in the development of obesity and type 2 diabetes. To determine the effect of exposure to DDE, trans-nonachlor, or oxychlordane on adipogenesis, NIH3T3-L1 preadipocytes were exposed to control media, vehicle (DMSO 0.1%), oxychlordane (1 or 10 uM), trans-nonachlor (1 or 10 uM), or DDE (1 or 10 uM) prior to and throughout differentiation. Upon maturation, cultures were subjected to Oil Red O staining to assess adipogenesis. Exposure to oxychlordane (10 uM) increased, DDE (10 uM) decreased, and trans-nonachlor had no effect on Oil Red O staining compared to vehicle. To determine the effect of exposure to oxychlordane or DDE on adipokine/cytokine production, mature adipocytes were exposed to vehicle (DMSO 0.1%), oxychlordane (1 or 10 uM), or DDE (1 or 10 uM) for 24 hours. Exposure to DDE significantly increased leptin and IL-6 concentrations in the culture media, concentrations of resistin, adiponectin, and MCP-1 remained unchanged, and TNFα levels were undetectable. Thus, exposure to oxychlordane or DDE may promote or inhibit adipose tissue expansion and obesity, respectively. In addition, exposure to DDE appears to stimulate the release of leptin and IL-6 which may contribute to the elevated systemic levels of IL-6 and hyperleptinemia that are commonly associated with obesity and type 2 diabetes.

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DOWN-REGULATION OF UREA CYCLE GENE EXPRESSION BY PFOA IN RATS.

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222 COMPARATIVE NEUROTOXICITY AND INTRA-CELLULAR ACCUMULATION OF FIVE POLYBROMINATED DIPHENYL ETHER (PBDE) CONGENERS IN MOUSE CEREBELLAR GRANULE NEURONS.

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Polybrominated diphenyl ethers (PBDEs), a group of flame retardants comprising 209 congeners, have become widespread environmental pollutants. High levels of PBDEs have been detected in human tissues, particularly in North America, and body burden is especially high in infants and toddlers, because of exposure through breast milk and house dust. Increasing evidence, provided by animal studies, suggests that PBDEs are developmental neurotoxins, though the underlying mechanisms are still unknown. Various PBDEs have been reported to cause oxidative stress and to induce apoptosis at cell death in several cell types. In the present study, we investigated the comparative neurotoxicity in mouse cerebellar granule neurons, of five BDE congeners, chosen among the most commonly found at the highest levels in human tissues. All BDE congener tested (BDE-47, -99, -100, -153, and -209) decreased cell viability and induced apoptotic cell death. They also caused oxidation and its methoxylated analogue 6-MeO-BDE-47) using the Ca2+-responsive dye Fluo-2/AM. For all end-points measured, the potency ranking of the congeners was BDE-100>BDE-47>BDE-99>BDE-153>BDE-209. Measurement of BDE congener levels in neurons after exposure to different concentrations showed a significant accumulation in cells, which followed the same relative ranking. The findings suggest that all BDE congeners tested exhibit the same general mode of action (inhibition of oxidative stress-mediated apoptosis), and that the ability of each isomer to elicit such effects is dependent upon their accumulation in neurons, particularly in mitochondria.

223 INVOLVEMENT OF CALCIUM-RELATED PROCESSES IN THE INHIBITION OF DEPOLARIZATION-EVOKED CALCIUM INCREASE BY HYDROXYLATED PBDES IN PC12 CELLS.

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Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) increase basal intracellular Ca2+ concentration ([Ca2+]i) by inducing release of Ca2+ from endoplasmic and mitochondrial Ca2+ stores. Considering the strong association of these intracellular Ca2+ stores as indicators of voltage-gated Ca2+ channels (VGCCs) for regulating Ca2+ homeostasis, effects on depolarization-evoked increase in [Ca2+]i were investigated in neuroendocrine pheochromocytoma (PC12) cells exposed to PBDEs (BDE-47, BDE-49, BDE-99, BDE-100, -153) and metabolites of BDE-47 (3-OH-BDE-47, 4-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, and 6-OH-BDE-153) and its methyalted congeners of BDE-49, BDE-99, and BDE-153 using the Ca2+-responsive dye Fluo-2/AM. PBDEs and 6-MeO-BDE-47 neither affect basal nor depolarization-evoked [Ca2+]i, with the exception of BDE-47, which increased fluctuations in basal [Ca2+]i, and moderately increased depolarization-evoked [Ca2+]i. After 20 min-pre-exposure, OH-PBDEs dose-dependently inhibit depolarization-evoked [Ca2+]i. This inhibition is potentiated by a preceding increase in basal [Ca2+]i, especially at high concentrations of OH-PBDEs. High increases in basal [Ca2+]i, strongly inhibit depolarization-evoked [Ca2+]i. A moderate inhibition of depolarization-evoked [Ca2+]i, was also observed for some OH-PBDEs when applied during depolarization. The present findings demonstrate that OH-PBDEs inhibit the increase in depolarization-evoked [Ca2+]i, which is potentiated by preceding increases in basal [Ca2+]i. Interestingly, inhibition of the increase in depolarization-evoked [Ca2+]i, appeared more sensitive to preceding increases in basal [Ca2+]i, by OH-PBDEs inducing mainly release of Ca2+ from intracellular stores compared to OH-PBDEs that induced also influx of extracellular Ca2+. This apparent difference in [Ca2+]i, close to the membrane suggests involvement of Ca2+-dependent regulatory mechanism in close proximity to the VGCCs.

224 ELUCIDATING THE MECHANISMS OF TOXICITY OF TRICHLOROETHYLENE METABOLITES.

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The EPA, Department of Defense and other regulatory agencies list trichloroethylene (TCE) and its metabolites as ‘emerging contaminants’ or chemicals of concern. TCE is well known a by-product of the chlorination of municipal water and is mainly used in industry as a solvent or degreaser. The mechanisms by which TCE exhibits its toxicity are vaguely understood. When TCE is oxidized via the cytochrome P450 pathway, metabolites such as dichloroacetic acid (DCA), trichlroacetic acid (TCA) and trichloroethanol (TCEOH) are formed. These metabolites are thought to be responsible for the toxic effects observed in past studies. There is overwhelming concern for the possible cancer and reproductive effects, particularly leukemia that TCE can induce. Investigating the mechanisms of toxicity will benefit the areas of toxicology, public health and occupational health and safety. Using the model system S. cerevisae and parallel deletion analysis can provide a better understanding of the pathways involved in TCE toxicity in yeast as well as higher organisms, such as humans. Previous studies with benzene and d-block metals have proven this as a successful tool in elucidating modes of action involved in toxicity and cancer. The goal of this project is to identify yeast genes essential for the response to three TCE metabolites (DCA, TCA, TCEOH) elucidate their possible mechanisms of action and identify homologs for these genes in higher organisms. IC50 values will be determined and will be subsequently utilized in the parallel deletion analyses to identify genes involved in response to the three metabolites. Preliminary results consisting of essential genes identified and possible modes of action will be reported. Future investigations will be conducted to both identify homologous genes in humans and investigate different TCE metabolites produced by the glutathione conjugation pathway. The results of this experiment will be used to gain an overall understanding of the mode of action(s) of trichloroethylene and to identify homologs and gene susceptibility in humans.

225 PFOA AND PFOS-INDUCED OXIDATIVE STRESS RESPONSE IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS.

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Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the two most widely perfluorinated chemicals (PFCs), which contain an eight-carbon backbone. They are man-made fluorocarbon-based acids that have been used in various industrial processes. They are non-biodegradable and persistent in the human body and environment. They are released directly from production processes, as well as from their use in manufacturing of new products. Human occupational and environmental exposure to PFOA and PFOS occurs globally. Accumulating surveillance data suggest associations between PFOA and PFOS exposures and adverse effects on human health, including lipid metabolism, uric acid metabolism, and reproductive risks. Whether these biomonitoring associations are etiologic remains a question. In this study, we demonstrated that exposure of human microvascular endothelial cells (HMVECs) to PFOA and PFOS induces the production of reactive oxygen species (ROS) at both high and low concentrations in a time-dependent manner. We have also found that PFOA exposure induces the production of ROS in a dose-dependent manner. Morphologically, we have found that exposure to PFOA and PFOS induces actin filament remodeling, endothelial permeability changes, and endothelial migration and in vitro enhancement of angiogenesis in HMVECs. Furthermore, we have demonstrated that the production of ROS plays a regulatory role in PFOA- and PFOS-induced actin filament remodeling and endothelial migration and permeability increase. Taken together, our results indicate that PFOA- and PFOS-induced ROS production may play a role in the alterations of endothelial permeability and migration. The results from this study may contribute to dissecting the molecular mechanisms involved in PFOA and PFOS toxicity.

226 THE ROLES OF ORGANIC ANION TRANSPORTERS IN RENAL ELIMINATION OF BRANCHED AND LINEAR PERFLUOROBUTYRATE IN RATS.

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Perfluorobutyrate (PFBA) is widely detected in the environment as a result of industrial uses. It has a much shorter serum half life compared to perfluorooctanoate (PFOA). In addition to the linear form (L-PFBA), there is only one branched form (B-PFBA) for this four carbon perfluorocarboxylate. Previous studies have shown that in the rat both L-PFBA and B-PFBA are mainly eliminated renally. Furthermore, B-PFBA was eliminated much faster than L-PFBA. Given the fact that both linear and branched PFBA are organic anions, we tested the hypothesis that organic anion transporters in the kidney may mediate the transport of L-PFBA and/or B-PFBA and contribute to the differences in their elimination patterns. We
transiently transfected HEK 293 cells with Oat1, Oat2, Oat3, Oatp1a1 or Uat1 constructs and measured the uptake of both linear and branched PFBA in comparison to HEK293 cells transfected with empty vector as negative control. LC-MS/MS analysis was used to determine intracellular PFBA concentrations. Uptake of B-PFBA into Oat1, Oat2, and Oat3 expressing cells was 1.5 to 2 folds over vector transfected control cells while uptake of into Uat1-expressing cells was 5 fold over vector control. None of these transporters was able to mediate the transport of L-PFBA. Oatp1a1 did not transport neither linear nor branched PFBA. In summary, of the five tested transporters, the basolateral Oat1 and Oat3 as well as the apical Uat1 and Oat2 can transport B-PFBA but not L-PFBA. In conclusion, the selective transport of B-PFBA by organic anion transporters may facilitate the active secretion of B-PFCA and contribute to the differences in renal elimination of L-PFBA and B-PFBA in rats.

TENUOUS DOSE-RESPONSE CORRELATIONS FOR COMMON DISEASE STATES: CASE STUDY OF CHOLESTEROL AND PERFLUOROOCANTANOATE/SULFONATE (PFOS/PFOA) IN THE C8 HEALTH PROJECT.

Persistent organic chemicals like PFOA/PFOS, dioxins, and PCBs pose a challenge to epidemiologic investigators because they lack an unexposed control group. To overcome this problem, outcome data in some studies are sorted by chemical dose level and findings in low-end dose groups are compared to sequential higher dose groups. An example of this is found in The C8 Health Project that evaluated serum PFOA/PFOS (C8) and total cholesterol among 46,294 West Virginia residents who lived, worked, or went to school for at least one year in a C8 contaminated water district and were over age 18 in 2005-2006. The risk for high total cholesterol (> 240 mg/dL) measured via odds ratios (OR) in logistic regression models showed sequential OR increases with PFOA quartile in comparison to the lowest quartile (OR = 1.00) that were each significantly elevated (OR = 1.21, 1.33, and 1.40, respectively), but age, sex, and body mass index were stronger correlates. Importantly, the magnitude of cholesterol increase was small (12 mg/dL from lowest to highest exposure decile) and comparison to similar statistics for the general U.S. population showed the C8 cohort had lower rates of high cholesterol. This suggests that inadvertent selection bias affected the lowest exposure quartile (control group). The shape of the PFOA dose-response curve, if meaningful, suggests a rapidly rising, asymptotic increased risk (by 40-50%) of high cholesterol which has not been observed in studies of high C8 exposure groups to date. Thus, these findings in the C8 cohort appear to be tenuous and require further evaluation for potential selection bias and inconsistency with other available findings. This case illustrates the substantial difficulties in assigning toxicological importance to statistical comparisons for common disease states that utilize subgroups with low exposures as the control group.

INHIBITION OF GAP JUNCTION INTERCELLULAR COMMUNICATION (GJIC) IN MOUSE LIVER CELLS BY TECHNICAL TOXAPHENE AND TOXAPHENE CONGENERS.

Chronic technical toxaphene (TT) exposure resulted in an increase in liver tumors in B6C3F1 mice. TT’s mode of action (MOA) appears to be on the tumor promotion process. To further evaluate this MOA, we examined the effects of TT, selected individual purified toxaphene congeners (P-26, P-50, P-62, Hx-sed and Hp-sed), and two mixtures of selected congeners, on mouse (male and female) primary cultured hepatocyte GJIC inhibition (a measure of tumor promotion activity). The selected congeners are important constituents of environmentally weathered TT. Controls consisted of phenobarbital (positive control) and the solvent (DMSO). TT was cytotoxic in a dose-and-time-dependent manner. Female mouse hepatocytes appeared to be more sensitive (low viability) than male hepatocytes after 3 and 8 h exposures, but not at 24 h. In the subsequent GJIC studies, we chose 5 μg/ml TT as the highest dose and two additional doses (1.0 μg/ml and 0.2 μg/ml) for both male and female mouse hepatocytes. After 3 or 24 h of treatment, TT at 5 μg/ml (but not at 1.0 μg/ml or 0.2 μg/ml) inhibited GJIC in a dose-dependent manner in both male and female mouse primary hepatocytes. All five congeners showed cytotoxicity, and they inhibited GJIC (at non-cytotoxic doses) with generally similar potency to TT. The two mixtures of congeners also inhibited GJIC, but required higher doses on a total w/v basis than either TT or the individual congeners. We conclude that TT, these 5 congeners and the two mixtures tested all have the potential to inhibit GJIC at doses below those causing cytotoxicity in male and female mouse primary hepatocytes. These observations provide additional support that tumor promotion is a likely MOA for the induction of B6C3F1 mouse liver tumors for all forms of toxaphene. This research was supported by a contract from Hercules Incorporated, a subsidiary of Ashland Inc.

SPECIES-SPECIFIC MULTIPlicity OF NUCLEAR RECEPTOR ACTIVATION AND METABOlISM REGULATION BY PERFLUOROOCANTANOATE (PFOA) AND PERFLUOROOCTAN SULFONATE (PFOS) IN CELL CULTURE.

PFOA and PFOS are environmentally persistent terminal breakdown products of perfluorooctane esters once widely marketed as water resist and non-stick surface applications. Although much of the biological activity of PFOA and PFOS in rodents is attributed to transactivation of the PPARα nuclear receptor, residual effects in PPARα null mice suggest potentially significant PPARα-independent effects in rodents and/or humans. To address this, we exposed rat or human primary hepatocytes in culture to 25 μM PFOA or PFOS for 24 hrs, and then using quantitative PCR, generated transcriptional profiles for both nuclear receptor activation and metabolic pathway regulation. Both PFOA and PFOS elicited a strong activation of PPARα− dependent gene transcription in rat liver cells; PPARα activation in human liver cells by either PFOA or PFOS was far less robust. Likewise, whereas PFOA and PFOS elicited a modest activation of CAR, LXR, FXR, and possibly PXR − dependent gene transcription in rat liver cells, activation of these alternate nuclear receptors in human liver cells was marginal. On a metabolic scale, transcriptional profiling suggests that PFOA and PFOS stimulate fatty acid oxidation and ketogenesis, but inhibit both glycolysis and ureogenensis in rat liver cells. Human liver cells did not exhibit such glycolytic, ketogenic or urea cycle changes in response to PFOA or PFOS exposure. The results demonstrate: 1) the multiplicity of nuclear receptors that participate in the transcriptional response to PFOA and PFOS in rat liver cells and, 2) the substantially decreased responsiveness of human liver cells to PFOA and PFOS induced transcriptional changes. What remains to be determined is whether activation of non-PPARα nuclear receptors in rodents reflects direct ligand activation by PFOA and PFOS, or do these reflect the generation of endogenous ligands secondary to the direct transactivation of PPARα or other nuclear receptor targets. (Supported in part by the 3M Co.).

QUANTITATIVE ASSESSMENT OF AEROSOLIZED CYANOBACTERIAL TOXINS IN TWO NEW ZEALAND LAKES.

The cyanobacterial toxins microcystin (MC) and nodularin (NOD) are highly effective inhibitors of cellular protein phosphatases. Toxicity primarily evolves following ingestion of cyanobacterial material or toxins and results in liver and renal pathology. Thus ingestion provides the main route of exposure in WHO’s current risk assessment of MC and NOD. However, nasally applied cyanotoxins appear to have a 10-fold higher availability and toxicity than oral applications, suggesting that aerosolized toxins could represent a major risk for humans inhabiting lake-sides with cyanobacterial blooms. Consequently, 12 and 24 hr MC and NOD levels in aerosols were assessed using high and low volume air samplers (HVS and LVS) at lakes Rotorua and Forsyth (New Zealand) which were experiencing dense blooms of cyanobacterial material or toxins and results in liver and renal pathology. These observations provide additional support that tumor promotion is a likely MOA for the induction of B6C3F1 mouse liver tumors for all forms of toxaphene.

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within a much shorter period may be underestimated. Indeed, following a simulta-
neously carried out single experiment with a 4 h sampling period, 0.64 ng MC filtr-
ter, corresponding to 4920 cells per filter, were detected despite this HVS having
been situated 20 m away from and 30 m above Lake Rotorua. Upon comparison to
the results of the simultaneously carried out 24 h sampling period, the latter sup-
ports the occurrence of extreme peaks of aerosolized cyanotoxin and toxic
cyanobacteria.

231 GENOME SCANS IN NATURAL POPULATIONS FOR
SELECTIVELY IMPORTANT SNPS USING HIGH-
THROUGHPUT TECHNOLOGY.
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Populations of the teleost fish Fundulus heteroclitus appear to flourish in heavily
polluted and geographically separated Superfund sites. In these polluted popula-
tions, natural selection likely has altered allele frequencies of loci that affect fitness.
The aim of this study was to use high-throughput sequencing and genotyping tech-
nology to identify and verify SNPs that exhibit non-neutral behavior in the F. hete-
rocitus genome in polluted populations versus clean reference populations. To de-
tect signatures of natural selection and identify genetic bias for adaptation to
anthropogenic stressors, we examined SNP allele frequencies for over 250 SNPs
among populations of F. heteroclitus. We contrasted populations from three
Superfund sites to clean reference populations flanking the polluted sites. This ex-
perimental design allows us to distinguish pollutant effects from demographic ones.
An FST modeling approach and an association test were used to identify SNPs ex-
hibiting non-neutral behavior. Among any one triad (polluted versus both reference
sites), approximately 11% of SNPs were identified as outliers in the FST test: 10
SNPs were shared among two of the triads, and three outlier SNPs were shared
among all three triads. Association testing identified on average 10% of
SNPs as outliers, of which 40% were shared outliers with the FST test. Two SNPs
were identified as outliers in both tests among all three triads strongly indicating
that they are under selection or linked to loci under selection in the polluted popu-
lations. These SNPs are located in the proximal promoter of cytochrome P450 1A
(CYP1A) and may play a functional role in CYP1A transcription. In the three
Superfund populations, CYP1A is refractory to induction by prototypical inducers.
In total, many SNPs were identified as outliers potentially under selection. These
outlier SNPs include two SNPs upstream of CYP1A. The role of these SNPs in the
refractory phenotype of CYP1A will be studied in the future.

232 INFLUENCE OF METABOLIC TRANSFORMATION
ON THE GILL AND LIVER OF RAINBOW TROUT
(ONCORHYNCHUS MYKISS) AND CHANNEL CATFISH
(ICATULARUS PUNCTATUS) ON BIOCONCENTRATION.
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University of North Texas, Denton, TX and 2Pfizer Global Research & Development,
Groton, CT. Sponsor: L. Harbell.
The potential for xenobiotic compounds to bioconcentrate is often expressed
through the calculation of the bioconcentration factor (BCF). BCF models tradi-
tionally have been single-compartment models with KOW as the primary input. A
multi-compartment model based on the principles of absorption, distribution, me-
tabolism and elimination (ADME) has been proposed given that several factors af-
fect the ability of a substance to bioconcentrate in an organism, including the abil-
ity of the organism to eliminate the compound through metabolic transformation.
In vitro biotransformation assays using S9 and microsomal fractions provide an
indication of metabolic potential. Traditionally, the liver is considered the primary
site of biotransformation. In the fish, however, the gill is considered a primary site
of xenobiotic uptake and has been shown to contain similar cytochrome P450 en-
zymes as the liver. To better understand the relative metabolism of xenobiotics in
the gill and liver, loss rate of parent was measured in gill and liver S9 and microso-
mal fractions. Test compounds included the pharmaceuticals ibuprofen and
nortriptyline, which are known to undergo microsomal biotransformation.
Additionally, glucuronidation of ibuprofen was also evaluated in S9 fractions.
Km values were then calculated and entered into a BCF model. A significant dif-
ference was noted between BCF solely based on KOW and BCF including KmET.
BCFs were further compared to in vivo whole fish BCFs. Thus, these studies indi-
cate that the inclusion of KmET in BCF models provide a more realistic indication
of bioconcentration potential. Furthermore, it is shown that the gill has metabolic
potential to eliminate certain compounds prior to systemic circulation in the fish.

233 POLYCHLORINATED BIPHENYLS DISRUPT
INTESTINAL INTEGRITY VIA NADPH OXIDASE-
INDUCED ALTERATIONS OF TIGHT JUNCTION
PROTEIN EXPRESSION.
Y. Choi1,2, M. J. Seelbach1, H. Pu2, S. Eum2, L. Chen2, B. Zhang3,2, B. Hennig1,3, M. Toborek1,2, 1College of Agriculture, University of Kentucky, Lexington, KY; 2Neurosurgery, University of Kentucky, Lexington, KY and 3Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY.
Polychlorinated biphenyls (PCBs) are widely distributed environmental toxica-
tants that contribute to numerous disease states. The main route of exposure to PCBs
is through the gastrointestinal tract; however, little is known about the effects of
PCBs on intestinal epithelial barrier functions. The aim of the present study was to
address the hypothesis that highly-chlorinated PCBs can disrupt gut integrity at the
level of tight junction proteins. Exposure of human epithelial Caco-2 cells to indi-
vidual PCB congeners, such as PCB153, PCB118, PCB104 or PCB126, resulted in
activation of NAD(P)H oxidase (NOX) and increased permeability of FITC-la-
beled dextran (4 kDa). Consistent with permeability results, treatment with PCB
congeners disrupted expression of tight junction proteins zonula occludens-1 (ZO-1)
and occludin. In addition, inhibition of NOX by apocynin significantly prote-
tected against PCB-mediated increase in epithelial permeability and alterations of
ZO-1 protein expression. Importantly, exposure to PCBs also resulted in alterations
of gut permeability via decreased expression of tight junction proteins in an intact
physiological animal model. These results indicate that oral exposure to highly-
chlorinated PCBs present a significant risk to intestinal epithelial integrity and may
directly contribute to the systemic effects of these toxicants.

234 SILICA NANOPARTICLES COATED WITH PCB153
ALTER THE BLOOD-BRAIN BARRIER INTEGRITY
AND INDUCE NEUROINFLAMMATORY RESPONSES.
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Of Neurosurgery, University of Kentucky, Lexington, KY and 3College Of Agriculture,
University of Kentucky, Lexington, KY.
Atmospheric transport is considered to be the primary pathway for global distribu-
tion of polychlorinated biphenyls (PCBs). In the atmosphere, PCBs are adsorbed
to aerosols with a particle size between 0.05 μm to 20 μm. To mimic these conditions,
we coated silica nanoparticles (20 μm diameter size) with PCB153. Mice
(C57BL/6 males, 12-week-old) were then injected with nanoparticle-coated
PCB153. PCB153 dissolved in 0.01% DMSO (PCB153/DMSO), nanoparticles
alone, or vehicle (DMSO or PBS). Sham control animals were subjected to surgical
procedures without additional treatment. All injections were performed through
the internal carotid artery and PCB153 was administered in the amount of 6 mg/g body
weight. Serum aspartate aminotransferase (AST) levels were elevated in mice
injected with PCB153-coated nanoparticles but not in mice injected with nanopar-
icles alone or PCB153/DMSO. Expression of tight junction proteins, ZO-1, oc-
cludin, and claudin-5, was altered in mice injected with PCB153-coated nanopar-
icles and with PCB153/DMSO. Importantly, exposure to PCB153-coated
nanoparticles but not to other treatment factors resulted in the loss of the integrity
of the blood-brain barrier (BBB) as determined by increased flux of sodium fluores-
cence from brain microvessels into the brain tissue. Exposure to PCB153-coated
nanoparticles also resulted in significant elevation of the mRNA levels of proin-
flammatory cytokines in the brain tissue. These results suggest that PCB153 and
nanoparticles can markedly increase their toxic effects. In addition, our data indi-
cate that PCB153-coated nanoparticles induce strong proinflammatory responses
in the central nervous system and can disrupt the integrity of the BBB via alter-
ations of tight junction protein expression.

235 MISEXPRESSION OF FGFI7B AND NOTCH1B ARE
ASSOCIATED WITH IMPAIRED HEART
REGENERATION IN ZEBRAFISH (DANIO RERIO)
EXPOSED TO 2, 3, 7, 8-TETRACHLORIDIBENZO-P-
DIOXIN (TCDD).
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Madison, WI.
TCDD is a global environmental contaminant that causes a wide spectrum of toxici-
ties in vertebrates. Although it is known that most toxic effects are mediated by
aryl hydrocarbon receptor (AHR) signaling, molecular mechanisms downstream of
AHR activation remain unclear. To gain insight into these mechanisms, we em-
ployed a zebrafish heart regeneration model. Our previous research found that

### 236 CYTOTOXICITY AND ESTROGENIC POTENTIAL OF PCB11 AND ITS METABOLITE 4-OH-PCB11.

S. Fleq, L. W. Robertson, H. Lehmler and G. Ludewig, of Occupational and Environmental Health, University of Iowa, Iowa City, IA.

Polychlorinated biphenyls (PCBs), a class of persistent organic pollutants (POPs), were commercially produced as different mixtures of the 209 individual congeners. The current PCB-contamination found in Chicago air strongly resembles a combination of two Aroclor mixtures. However, one of the detected main congeners, 3,3’-dichlorobiphenyl (PCB 11), is not a constituent of these commercial mixtures. It turns out that this compound is ubiquitous in air throughout the city of Chicago, in the Delaware River, and the New York/New Jersey Harbor. It is currently hypothesized that the main source of PCB11 is not from declorination of higher chlorinated congeners, but from the production, use and/or deterioration of dichlorobenzidine-based yellow pigments in paints and resins. Besides its volatility and occurrence in inner city air, very little is known about the toxicological properties of this PCB congener, but it is assumed to be easily biotransformed through cytochrome P450s to possibly more reactive metabolites. To fill this gap in our knowledge, we analyzed the cytotoxicity of PCB11 and its metabolite 4-OH-PCB11 in different cell lines and measured their potential estrogenic effects in comparison to other PCB congeners and a synthetic Chicago air mixture using MCF-7 cells. In most tested cell lines PCB11 was only cytotoxic at relatively high concentrations (EC50 ≥ 100 μM) after 24-72 h of exposure in media with 10% fetal bovine serum (10% FBS-medium). 4-OH-PCB11 was slightly more toxic (EC ~ 50-100 μM) after 24 h exposure. Cytotoxicity of each compound increased if 1% FBS-medium was used (EC ~ 10-25 μM). In the E-screen assay with 5% Dextran-coated charcoal-treated FBS (DCCFBS), 4-OH-PCB11 was more potent than the parent compound PCB11; also active were the commercial PCB mixtures Aroclor 1242 and Aroclor 1254 and the synthetic Chicago air mixture. These results suggest that this non-Aroclor congener and its 4-OH metabolite probably have low acute toxicity, but may act as weak endocrine disruptors. (Supported by NIEHS P42 ES013661, ES05605 and DOD DAMD17-02-1-0241)

### 237 INHALATION EXPOSURE OF RATS TO A PCB MIXTURE RESEMBLING THE CHICAGO AMBIENT AIR CONGENER PROFILE.

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Despite the continued occurrence of semi-volatile PCBs in the atmosphere, there have been few toxicology studies that investigated the inhalation route of exposure. We developed an inhalation exposure system capable of generating controlled atmospheres of vapor-phase PCB mixtures with a congener profile of the Chicago airshed. We sought to measure the uptake and toxicological responses in Sprague-Dawley rats. We generated vapor-phase PCBs under carefully-controlled conditions into a moving airflow that was then diluted and fed to a nose-only exposure chamber. Chamber outflow was sampled using XAD cartridges replaced daily and characterized for congener profiles using GC/MS/MS. Rats were exposed 2 hr/day for 10 days via nose-only inhalation. Pulmonary and immune responses were evaluated from bronchoalveolar lavage fluid and histopathology of lungs, trachea, nose, nasopharynx and thymus. Lungs, livers and blood were collected to measure PCB uptake in tissues 24 hr post exposure. Exposure data showed that a very high dose could be achieved with a high degree of consistency in concentration and PCB congener profile distribution. In a high dose experiment, we generated 11.5±2.0mg/kg/day total PCBs (mean ± SD) during the 2hr daily exposure period. The low molecular weight PCB congeners (mono-, di- and trichlorinated) represented 90% of the total PCBs in the atmosphere. PCB 1, 4, 6, 8, 15, 17, 18, 20, 24, 28, 31, 32, and 52 were most abundant by mass, accounting for 83±4% of the total PCBs. We found the largest amounts of PCBs taken up in rat liver, lower in the lung and the lowest in the blood. PCB 20, 24, 49±69, 52, 60, 61±79±74, 76, 66, 83±99±112, 85±116±117, 90±10113, 103, 118 were leading congeners in the three tissues. The toxicology evaluation showed that there was minimal lung inflammation and little evidence of lung injury.

### 238 OXIDATIVE DNA ADDUCTS IN THE LIVERS OF FEMALE SPRAGUE-DAWLEY RATS CHRONICALLY EXPOSED TO POLYHALOGENATED AROMATIC HYDROCARBONS (PHAHs).

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Oxidative DNA damage induced by free radical species and the resultant lipid peroxidation products are regarded as a likely contributors to the carcinogenesis of non-genotoxic compounds, especially PHAHs. Because of their resistance to degradation, widespread distribution in the environment and bioaccumulation in organisms, these compounds lead to chronic exposures in humans and animals, which can result in toxicity and carcinogenesis. The present study examined several key oxidative DNA lesions in livers of female Sprague-Dawley rats exposed to PHAHs for 53 weeks. The results demonstrated that co-exposure of female rats to PCB 153 and 126 induced a synergistic effect on the induction of 8-0HdG and MIG. PCB153, which does not have a TEF, also induced increases in 8-0HdG and N2,3-εG, 8-0HdG, N2,3-εG and MIG showed dose-dependent increases after exposure to the binary mixture of these PCBs. For rats exposed to TCDD and the tertiary mixture of TCDD, PCB126 and PCDF, the shape of the dose-response curve for 8-0HdG was different from 8-0HdG, N2,3-εG, and 7OEG. Differences also existed for oxidized DNA adducts in female rats exposed to the same TEF dose of TCDD and the tertiary mixture. Higher amounts of 8-0HdG were detected in the animals exposed to the tertiary mixture than TCDD with the same TEF dose, but lower amounts of N2,3-εG and 7OEG were detected in the same samples. The above results suggest that oxidative DNA damage contributes to the toxicity of PHAHs and is not completely dependent on the aryl hydrocarbon receptor. Cautionous use of TEF values is needed when evaluating the oxidative DNA lesions and comparing the dose response behavior of DLCs or their mixtures.

### 239 DIOXIN-LIKE PCB126 (3, 3’, 4, 4’, 5- PENTACHLOROBIPHENYL) INCREASES RAT PON1 (PARAOXONASE 1) ACTIVITY: AN AHR (ARYL HYDROCARBON RECEPTOR) - MEDIATED PATHWAY?

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PON1 (Paraoxonase 1), an HDL (High Density Lipoprotein)-associated antioxidative protein, is primarily synthesized in the liver and secreted into the bloodstream. It displays preventative properties against cardiovascular diseases mainly through removal of oxidized phospholipids in low density lipoproteins and cell membranes. Serum PON1 levels are modified by genetic and environmental factors. For example, an XRE- (Xenobiotic response element) like sequence (Gouédard et al., 2004) located in the PON1 gene promoter region was reported to bind to the AhR; the AhR activation depended on the aryl hydrocarbon receptor. Cautious use of TEF values is needed when evaluating the oxidative DNA lesions and comparing the dose response behavior of DLCs or their mixtures.

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**240 MODULATION OF MNSOD EXPRESSION BY PCB126 OCCURS AT MULTIPLE LEVELS.**

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Manganese superoxide dismutase (MnSOD) is one of the most efficient antioxidant enzymes. Previous studies found that 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD), the most potent aryl hydrocarbon receptor (AhR) agonist, decreased MnSOD activity in rats, thereby rendering the animals more vulnerable to oxidative stress. However, the exact mechanisms and whether it is an AhR-related is still unclear. To explore this issue, the impact of PCB126 (3',3',4,4',5-pentachlorobiphenyl) on MnSOD expression was studied. PCB126 is the most efficient AhR agonist in the family of polychlorinated biphenyls capable of causing sustained AhR activation and a variety of serious liver conditions. Male Sprague Dawley rats were treated with a single injection of PCB126 at different doses. Twelve weeks later their livers were harvested and MnSOD expression determined together with liver Mn content. The results indicate an increase in MnSOD mRNA levels, a decrease in enzyme activity, which is consistent with TCDD's effect, and no change in protein levels, suggesting that the regulation of MnSOD by PCB126 occurs at different levels, probably including the AhR. In addition, liver manganese levels were decreased by as much as 33%. Subsequent in vitro mechanistic studies using a rat hepatoma cell line failed to reproduce the effects on MnSOD expression. Supplementation of the culture medium with manganese, to eliminate manganese-related changes and apoptosis were observed at high (≥0.2 mM) manganese concentrations. The discrepancy between in vivo and in vitro studies awaits further investigation, the results of which should add to our overall knowledge about the regulation of this important protective enzyme and about mechanisms of PCB126's toxicity, thus providing insight into developing possible means for chemoprotection. 

(Supported by NIEHS P42 ES013661, ES05605, and DOD DAMD17-02-1-0241)

**241 UV-INDUCED TOXIC EFFECTS OF A BROMINATED DIPHENYL ETHER (DECA-BDE) AND A HYDROXYLATED CHLORINATED DIPHENYL ETHER (TRICLOSAN) IN HUMAN SKIN CELLS.**

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The flame retardant decabromodiphenyl ether (deca-BDE) and the bactericide triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) are widely used in consumer products such as carpets and skin care items, respectively. Dermal contact is a major exposure route for both chemicals. Our skin is inevitably exposed to UV light. Knowing deca-BDE and triclosan are degraded by UV irradiation, we hypothesize that combined exposures to halogenated diphenyl ethers and its hydroxyl compounds (deca-BDE and triclosan) in skin cells with the resazurin assay. Immortalized human keratinocytes (HaCat) and human skin fibroblasts (HSF) were treated for 1 h with deca-BDE in PBS and/or 25, 50 and 100 μM/cm2 UVB + UVA. After 24 h treatment in DMEM, 25 μM deca-BDE + UVA caused synergistic cytotoxicity in HaCaT. In HSF, synergistic cytotoxicity was visible with 6.3, 12.5 and 25 μM deca-BDE + 100 μM/cm2 UV. 100 μM triclosan was irradiated in PBS (pH 7.4) with 1 J/cm2 UVB + UVA. Irradiated and non-irradiated triclosan were diluted 20-fold with F12/DMEM (3:1) medium and added to HaCaT cells. After 24h incubation with irradiated triclosan, less than 50% of cells were viable while over 90% of cells exposed to non-irradiated triclosan survived. The 3950 nm absorbance spectrum of triclosan was shifted to longer wavelengths and the intensity was significantly increased after UV irradiation, suggesting the production of degradation products like dioxins and smaller phenols. The toxic effects of irradiated triclosan are most likely due to these degradation products. Overall our results support the hypothesis that co-exposure to halogenated diphenyl ethers or their hydroxyl derivatives and solar UV light significantly increases their toxicity, a finding that may have important implications for our use of these consumer products. (Supported by NIEHS P42 ES013661 and U.S. EPA 05605, DOD DAMD17-02-1-0241, EPA R-82920120-0, and CHEEC).

**242 CHOLESTEROL INHIBITION AND URINARY METABOLITE LEVELS FROM REPEATED EXPOSURES TO A MIXTURE OF TWO ORGANOPHOSPHORUS INSECTICIDES IN RATS.**

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Exposure to multiple organophosphorus (OP) insecticides, which inhibit cholinesterase (ChE), is high. Biomonitoring studies have detected urinary OP metabolites in large numbers of people. The development of a reverse dosimetry mathematical model could predict the exposure scenario through the use of biomarkers such as urinary metabolites or blood ChE inhibition data. Rats were exposed to chloropyrifos (CPS) and diazinon (DZN), alone or in mixtures. Every 4 days over a 15 day period, rats were exposed orally to CPS (7.5mg/kg) or DZN (60mg/kg) or a mixture of the individual dosages or an equal mixture of the individual dosages. Brain, plasma and red blood cell (RBC) ChE and liver and serum carboxylesterase (CaE) activities were determined spectrophotometrically. Urine was collected for 24 hr intervals for metabolite detection by gas chromatography/mass spectrometry. ChE inhibition was dose related, with peak inhibition in the brain of about 20% for the individual compounds and the lower dose mixture, and about 60% in the higher dose mixture 24 hr after exposure. Plasma and RBCs showing greater inhibition than the brain. ChE activity recovered partially prior to subsequent exposure, so there was a small increase in inhibition with repeated exposures. The metabolites trichloropyridinol (TCP; for CPS) and 2-isopropyl-4-methyl-6-hydroxyprymidine (IMHP; for DZN) were largely excreted in the first 24-48 hr following each exposure. TCP levels were slower to return to baseline. These data sets indicate that DZN is metabolized relatively fast and CPS, a more lipophilic compound, slightly slower but showed no real interaction for the two OPs. The small increases in ChE inhibition with repeated exposures are the result of a lack of recovery of activity and not the extended presence of insecticide. (Supported by EPA STAR grant RD 83345101)

**243 DRUGS POTENTIALLY AFFECTING TRICHLOROETHYLENE METABOLISM.**

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Trichloroethylene (TCE) is a volatile solvent to which humans are frequently exposed. Toxicokinetic interactions on TCE have been reported with aspirin and acetaminophen but no information can be found for other drugs. The purpose of this study is to identify drugs that could potentially affect the metabolism of TCE. Fifteen widely used drugs were selected: acetaminophen, ibuprofen, acetylsalicylic acid, mefenamic acid, naproxen, diclofenac, sulphasalazine, cimetidine, ranitidine, valproic acid, carbamazepine, amoxicillin, erythromycin and gliclazide. Suspensions of rat hepatocytes were exposed to TCE alone and in presence of each of these drugs (10x therapeutical maximal levels) in closed vials. The concentrations of TCE and its metabolites trichloroethanol (TCHO) and trichloroacetic acid (TCA) were measured by headspace gas chromatography coupled to mass spectrometry (GC-MS). According to results, drugs could be separated into three groups. Group 1 is composed of drugs causing no significant interactions (i.e., Carbamazepine, ibuprofen, mefenamic acid and ranitidine). Group 2 includes drugs significantly affecting both TCHO and TCA levels: decreased levels by valproic acid (3-fold), acetaminophen (2-fold) and gliclazide (2-fold); increased levels by acetylsalicylic acid (1.5-fold) and naproxen (2-fold). While decrease in metabolite levels may be explained by inhibition of CYP activity, increases may result from inhibition of glucuronidation. Finally group 3 includes drugs that affect either TCHO or TCA levels. TCA levels were decreased by cimetidine, diclofenac, sulphasalazine and amoxicillin, whereas erythromycin decreased TCHO concentrations. Our results confirm the existence of interactions between TCE and a variety of widely used drugs. These interactions can result in the modulation of internal levels of one or even two metabolites. Further studies in human material are needed to assess implications for human health. Funded by Natural Sciences and Engineering Research Council of Canada.
PCB153, a mixture of 30 Immature, ovariectomized mice were gavaged with 30 μg/kg TCDD and PCB153, the most abundant non-dioxin-like PCB, were examined. Exposures to environmental contaminants, such as dioxins and PCBs, frequently occur as complex mixtures. In this study, the hepatic effects elicited by a mixture of TCDD and PCB153, the most abundant non-dioxin-like PCB, were examined. Immature, ovariectomized mice were gavaged with 30 μg/kg TCDD, 300 mg/kg PCB153, a mixture of 30 μg/kg TCDD and 300 mg/kg PCB153 (MIX) or sesame oil vehicle for 4, 12, 24, 72 or 168 h. Significant increases in relative liver weights (RLW) were induced with TCDD (24-168 h), PCB153 (72-168 h) and MIX (24-168 h). MIX elicited significantly greater increases in RLW, accompanied by marked hepatocellular hypertrophy when compared to TCDD or PCB153 alone. Complementary histopathology (Oil Red O), gas chromatography/mass spectrometry and triglyceride analyses identified the most significant increases in lipid levels in the MIX group at 24 hrs. TCDD also caused a dramatic increase in lipid content at 72 and 168 h, while PCB153 elicited no significant changes in lipid levels. Hepatic gene expression profiling with Agilent whole-genome microarrays identified 573 unique gene expression changes, with 170, 186 and 391 differentially regulated in response to TCDD, PCB153 and MIX, respectively ([fold change]>1.5, P1(<0.001). MIX elicited a wide range of putative non-additive effects compared to TCDD and PCB153, consistent with putative non-additive increase in hepatic lipid accumulation and hepatocellular hypertrophy. Moreover, hepatic PCB153 levels were significantly increased with TCDD co-exposure at 24 and 168 h. Collectively, these results suggest there are non-additive interactions between TCDD and PCB153 that lead to non-additive effects on RLW, hepatocellular hypertrophy and fat accumulation, gene expression and PCB153 tissue levels. Funded by SBRP P42ES09111.

Disinfection of water greatly decreases waterborne disease. Disinfection byproducts (DBPs), including haloacetic acids (HAAs), are formed when oxidizing disinfectants react with inorganic and organic matter in water. Water providers routinely assay for 9 HAAs in finished drinking water: chloro-, dichloro-, trichloro-, bromo-, dibromo-, tribromo-, bromochloro-, bromodichloro- and dibromochloro-acidic acid. Iodoacetic acid (IA) is a recently identified DBP. We assessed the validity of an assumption of dose addition for 4 HAAs mixtures: environmentally relevant mixtures of the 9 HAAs combined at mixing ratios representative of postchlorination (CL) and preozonation/postchlorination, as well as equimolar mixtures of the 9 common HAAs (9HAA EQM) and a 10 HAA mixture (10HAA EQM) comprised of the 9 common HAAs and IA. The individual HAAs and the HAA mixtures were evaluated in a Chinese Hamster Ovary cell chronic cytotoxicity assay at a minimum of 10 concentrations each. The individual HAA data were used to develop a smooth additivity model consistent with Berenbaum’s definition of additivity. The fitted models provided reasonable fits to the single chemical data. The slope of the fitted dose-response curve for each individual HAA and HAA mixture was negative and statistically significant, indicating that cell density decreased significantly with increasing concentration. The overall test for departure from dose additivity was significant (p<0.001) for each mixture. Individual dose levels of each mixture (with Hochberg’s correction applied, alpha=0.05) demonstrated less-than-additive (anagonistic) departures from additivity in the higher portions of the mixture dose-response curves. Deviation from dose additivity was not detected at the lowest 2 (10HAA EQM), 3 (9HAA EQM) or 4 (CL, OZ-CL) tested concentrations of each mixture. In summary, all 4 HAA mixtures exhibited dose-dependent less-than-additive toxicity. (May not reflect EPA policy. Funded by EPA.)
tested for toxicity alone. Concentration-response data, obtained from seven concentrations and a control (each duplicated), were fit to sigmoid curves using a four-parameter logistic function. Soft electrophiles tested included agents reactive via addition (e.g., Michael-type) or substitution (e.g., SN2 and SNAR) and these agents were tested in sham and true mixtures and/or in combination with a non-reactive, non-polar narcotic. Since reactivity rate (e.g., very fast to very slow) appears to affect TDT level (e.g., low, moderate, high, full), both factors affect mixture toxicity. Data analysis gave additivity quotient (AQ) and independence quotient (IQ) values in which an EC50-AQ or EC50-IQ value near 1.0 represents dose addition or independence, respectively. Additional parameters used were the slopes of concentration-response curves, relative potency of the mixture agents, and EC25/EC75-AQ or IQ values. To date over 100 combinations have been tested. Despite the confounding factors of varying reactivity rates and TDT levels the results support the idea that a dose-additive combined effect is restricted to agents inducing toxicity through a single common mechanism of action rather than via a common mode of toxicity. As expected, most combinations show a combined effect fairly close to dose-additive, as instances of strong synergism or antagonism were rare.

249 MODELING THE INTERACTION OF BINARY AND TERNARY MIXTURES OF ESTRADIOL AND BISPHENOL A OR ITS ANALOGUES IN AN IN VITRO ESTROGEN MEDIATED TRANSCRIPTIONAL ACTIVATION ASSAY (T47D-KBLUC).

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Bisphenol A is a ubiquitous monomer used to manufacture polycarbonate plastics. Exposure of human and wildlife populations to bisphenol A and its analogs is widespread and well documented. Bisphenol A is hypothesized to be estrogenic in both in vivo and in vitro studies and has been shown to bind to estrogen receptors α and β to induce estrogenic activity via estrogen response elements. The current study tests binary and ternary mixtures of an endogenous estrogen (estradiol-17β) with bisphenol A or its analogues tetrabromo-bisphenol A or bisphenol AF and evaluates their interaction across a range of concentrations to determine if the mixtures emulate a dose-addition model of interaction. The T47D-KBLuc estrogen-responsive transcriptional activation luciferase reporter gene assay was used to determine a full dose-response and obtain EC50 and HillSlope data for the mixture chemicals tested. EC50 and HillSlope data are as follows: estradiol-17β, 5.47±1.3, 0.7542; bisphenol A, 7.22±0.08, 1.108; and bisphenol AF, 2.04±0.08, 1.106. Tetrabromo-bisphenol A induced no estrogenic dose response on the T47D-KBLuc assay and no dose-response and obtain EC50 and HillSlope data for the mixture chemicals tested. The T47D-KBLuc estrogen-responsive transcriptional activation luciferase reporter gene assay was used to determine a full dose-response and obtain EC50 and HillSlope data for the mixture chemicals tested. EC50 and HillSlope data are as follows: estradiol-17β, 5.47±1.3, 0.7542; bisphenol A, 7.22±0.08, 1.108; and bisphenol AF, 2.04±0.08, 1.106. Tetrabromo-bisphenol A induced no estrogenic dose response on the T47D-KBLuc assay and no dose-response and obtain EC50 and HillSlope data for the mixture chemicals tested.

250 IN VITRO - IN VIVO EXTRAPOLATION OF THE HUMAN DOSE-RESPONSE RELATIONSHIP FOR CELLULAR PERTURBATIONS BY A BINARY MIXTURE OF TOLUENE (TOL) AND N-HEXANE (HEX).

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The objective of this study was to predict the in vivo dose-response relationship in humans based on in vitro concentration-response data for chemical mixtures. For this purpose, the in vitro study in which Jurkat-T cells were exposed sub-chronically to TOL and HEX (McDermott et al. 2008. Toxicol. Sci. 101:263-274) was selected. The approach involved: (i) computation of the cellular concentrations of individual chemicals based on the incubation medium concentration in vitro, and (ii) determination of the inhalation concentrations of TOL and HEX, yielding these cellular concentrations in vivo at steady-state, using a previously published human physiologically-based pharmacokinetic (PBPK) model for this chemical mixture. The concentration of TOL and HEX in the cells (i.e., human T-lymphocytes) in vitro was estimated from knowledge of the concentration in culture medium and the lymphocyte:medium partition coefficient (PC). The concentration of TOL and HEX in lymphocytes in vivo was computed by incorporating lymphocyte blood PC in the PBPK model. These PCs were estimated on the basis of the cellular and blood content of the lipids and water, as well as Kow of TOL and XYL. The results indicated that, for TOL alone, in vitro exposure concentrations of 3.77, 5.09 and 10.2 μM would correspond to in vivo (inhaled) concentrations of 105, 125 and 193 ppm, respectively. Similarly for HEX, the in vitro exposure to 1.22, 2.04 and 4.08 μM would compare with continuous human inhalation exposure to 60, 100 and 195 ppm. In the case of mixed exposures, for LDH leakage and intracellular Ca++ perturbations, the lowest concentrations at which supra-additive effects were observed in vitro (1.22 μM HEX and 3.77 μM TOL) would correspond to in vivo co-exposures of humans to 58 ppm HEX and 87 ppm TOL, as determined with the interaction-based PBPK model. Overall, this study demonstrates the use of human PBPK models in the conduct of in vitro-in vivo extrapolation of the dose-response relationship for mixtures obtained in high throughput assays.

251 COMBINED EFFECTS OF DIRECT-ACTING MICHAEL ACCEPTOR COMBINATIONS VARY DEPENDING ON REACTIVITY RATES AND TIME-DEPENDENT TOXICITY OF THE AGENTS.

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Microtox was used to evaluate the combined effects of binary mixtures of direct-acting Michael acceptors. Four agents: p-benzoquinone (pBQ), 2-hydroxyethyl acrylate (2HEA), diethyl maleate (DEM), and hydroxypropyl methacrylate (HPM) were tested for reactivity with glutathione. Each agent was also tested for toxicity with each other at 15, 30 and 45 minutes. Data from seven duplicated concentration-Time-Delay (TDT) levels for each single agent and mixture were fit to sigmoid curves using a four-parameter logistic function. Data analysis gave time-dependent toxicity (TDT), and additivity quotient (AQ) or independence quotient (IQ) values, for which an EC50-AQ or EC50-IQ value of about 1.0 represents dose-addition or independence, respectively. Final determinations of combined effects were aided by relative reactivity rates provide information relevant to TDT; while slope and AQ/IQ values examine fit of experimental curves to predicted curves at other than the mid-point. TDT levels (pBQ–none, 2HEA–high, DEM–moderate, HPM–low) and relative reactivity rates (pBQ–very fast, 2HEA–fast, DEM–slow, HPM–very slow) varied among the four agents, thereby affecting mixture toxicity. Each pBQ-containing combination was less-than-dose-additive (EC50-AQs above 1.18). For 2HEA-DEM (1.05-1.10) and 2HEA-HPM (0.95-0.98), EC50-AQ values were closer to dose-additive. DEM-HPM EC50-AQ values were greater-than expected for dose-addition (0.81-0.87). The results can best be explained by the multiple mode of toxic action concept, that when toxicity of an agent is not fully time-dependent the agent exerts two toxic actions – one due to narcosis and the other due to its reactivity with nucleophiles within cells, such as enzymes.

252 MIXTURE TOXICITY AND MULTIPLE MODES OF TOXIC ACTION: DIRECT-ACTING MICHAEL ACCEPTORS WITH A NON-POLAR NARCOTIC.

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Recent Microtox studies on mixture toxicity of soft electrophiles have noted that the combined effect observed is affected by chemical reactivity, which affects its time-dependent toxicity (TDT). For slowly reacting agents, narcosis and reactive toxicity may both occur, giving multiple modes of toxic action. To study this further, four direct-acting Michael acceptors: p-benzoquinone (pBQ), 2-hydroxyethyl acrylate (2HEA), diethyl maleate (DEM), and hydroxypropyl methacrylate (HPM) were tested for reactivity with glutathione to determine relative reactivity rates. Each was tested for toxicity with the non-reactive, non-polar narcotic 3-methyl-2-butanone (5MB2). Acute toxicity was assessed at 15, 30 and 45 minutes of exposure, with seven concentrations and a control (each duplicated). Concentration-response curves were fit to sigmoid curves using a four-parameter logistic function. Data were used to obtain time-dependent (TDT) values and both additivity quotient (AQ) and independence quotient (IQ) values, wherein an EC50-AQ or EC50-IQ value of near 1.0 suggests dose-additive or independent combined effects, respectively. In addition to chemical reactivity, slopes of concentration-response curves and EC25-AQ (or IQ)/EC75-AQ (or IQ) values aided combined effects assessments. The very fast- (pBQ) and fast- (2HEA) reacting agents tested with 3MB2 resulted in combined effects that were less-than-dose-additive (i.e., EC50-AQ values from 1.10-1.22). The slow-reacting agent (DEM) gave an EC50-AQ value closer to dose-additive at 15-min (1.06), but this increased over time (1.13-1.21) as reactive toxicity became more prominent. For the very slow-reacting agent with 3MB2, dose-additive toxicity at 15-min (0.93) became greater-than dose-additive at 30 (0.89) and 45 min (0.85).
253 COMPARISON OF MIXTURE TOXICITY FOR MONO-, DI-, AND TRI-HALOGENATED ACETONITRILE COMBINATIONS.

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Halogenated acetonitriles were tested alone and in binary mixtures using Microtox to assess relationships between chemical structure and combined effect. Derivatives tested included mono (isodo, bromo, chloro), di (dibromo, dichloro) and tri (trichloro) forms. Toxicity was assessed at 15, 30 and 45 minutes of exposure with seven concentrations (each at four replicates) and analyzed with a logistic model (each a control indicating the absence of any toxic effect) or as a combination of each and the respective mixture. The results of experiments showed that toxicity was significantly increased in the case of mixtures compared to the single forms. A general multiplicative model was used to compare toxicities. The binomial probabilities of toxicity were analyzed using linear regressions. The results of the analysis showed that the toxicities of mixtures were significantly higher than those of single forms. The study provides valuable insights into the synergistic effects of halogenated acetonitriles, which are important for understanding the environmental impact of these compounds.

254 HEATH-TREATED PM10 RETAIN A NON-VOLATILE COMPONENT RELATED PRO-OXIDATIVE POTENTIAL.

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Current working hypothesis on the mechanisms mediating particulate matter (PM) toxicity resides on its capacity to induce oxidative stress on cells. PM metals and organics have the potential to cause oxidative stress. However, further understanding of the mechanisms behind PM toxicity is needed. As part of the MILAGRO campaign in Mexico City, we identified that PM10 pro-oxidative potential did not correlate with the PM components identified by EPR as having oxidative potential. To further understand the process, we expanded our study subjecting aliquots of the same PM samples to different temperatures (22, 50, 100, 150 and 175°C, 30 min) in order to evaporate volatile components. PM was sampled daily during March 2006 on cellulose membranes using High Vol samplers. Daily samples were pooled after being physically removed from the membranes. After temperature treatment, samples underwent Electron Paramagnetic Resonance (EPR) analysis (JEOL-JES-TE-300) in the absence (CF 328 mT, 9.4 GHz, 10 mW, and 175°C /30 min) in order to evaporate volatile components. PM was sampled to assess relationships between chemical structure and combined effect. Derivatives tested included mono (isodo, bromo, chloro), di (dibromo, dichloro) and tri (trichloro) forms. Toxicity was assessed at 15, 30 and 45 minutes of exposure with seven concentrations (each at four replicates) and analyzed with a logistic model (each a control indicating the absence of any toxic effect) or as a combination of each and the respective mixture. The results of experiments showed that toxicity was significantly increased in the case of mixtures compared to the single forms. A general multiplicative model was used to compare toxicities. The binomial probabilities of toxicity were analyzed using linear regressions. The results of the analysis showed that the toxicities of mixtures were significantly higher than those of single forms. The study provides valuable insights into the synergistic effects of halogenated acetonitriles, which are important for understanding the environmental impact of these compounds.

255 PHYTOTOXICITY OF METAL OXIDE NANOPARTICLES TO ARABIDOPSIS THALIANA.

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The nanotechnology industry is projected to be worth approximately 2.6 trillion dollars by 2015. This estimation has escalated the concern over the potentially toxic effects of the nanoparticles, causing the recent advent of nanotoxicology. Thus far, only a few studies have considered the toxicity of nanoparticles to plants. Phytotoxicity of nanoparticles is important to fully understand the potential environmental impacts of manufactured nanomaterials. The present study reports on the effects of four metal oxide nanoparticles, aluminum oxide (nAl2O3), silicon dioxide (nSiO2), magnetite (nFe3O4), and zinc oxide (nZnO), on the development of Arabidopsis thaliana (Mouse-ear cress). Three toxicity indicators (seed germination, root elongation, and number of leaves) were quantified following exposure to each nanoparticle at three concentrations (400, 2000 and 4000 mg/L) for 18 days. Among these particles, nZnO was most phytotoxic, followed by nSiO2, nZnO, and nAl2O3, respectively. Consequently, nZnO was selected for further experiments to determine the importance of particle size and zinc dissolution as toxicity determinants. Soluble zinc concentrations in nanoparticle suspensions were nearly 33-fold lower than the minimum inhibitory concentration of dissolved zinc salt (ZnSO4), indicating that zinc dissolution could not solely account for the observed toxicity. In addition, the inhibition of seed germination by ZnO depended on particle size with nanoparticles exerting higher toxicity than micron sized particles at equivalent concentrations. Overall, the present study demonstrates possible adverse effects of metal oxide nanomaterials on plants and calls for further research on the potential impacts of manufactured nanoparticles on agricultural and environmental systems.

256 INDUCTION OF ENDOTHELIAL DYSFUNCTION BY IRON OXIDE NANOPARTICLE EXPOSURE.

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The correlation between nanoparticle exposure and cardiovascular diseases attracts much concern over the environmental-health effects of nanotechnology. Nanoparticle triggered endothelial dysfunction is hypothesized to be an important mechanism. In the present study, nFecO3 and nFecFeO3 nanoparticles were selected to test this hypothesis and approach the biological pathways. Nanoparticles expose to human aortic endothelial cells (HAECs) and monocyte-macrophages (U937 cells) in the separate culture or co-culture system were applied to study the direct effects on HAECs and the indirect effects mediated by macrophage activation. The endothelial cell viability, membrane permeability and several crucial parameters involved in endothelial dysfunction were evaluated. Our results indicated exposure to iron oxide nanoparticles could induce HAECs vacuolization and cell death. Significant increase of HAECs membrane permeability and nitric oxide (NO) production were induced by activated-macrophages mediation. The monocyte adhesion to endothelial cells was enhanced associating with the up-regulation of intracellular cell adhesion molecule-1 (ICAM-1) and interleukin-8 (IL-8) expression. Iron oxide could be dissolved by macrophage phagocytosis. The overloading of phagocytosis activated macrophages and provoked oxidative stress. It is hypothesized that the intravascular iron oxide nanoparticles could affect the endothelial system by the nanoparticles, the released iron ions by phagocytosis, and the activated macrophage mediation. The induction of endothelial inflammation and dysfunction by iron oxide nanoparticles exposure were considered to be significant risk factors in atherogenesis.
phospholipids were demonstrated in the BAL fluid at 10 days post exposure in CeO$_2$-exposed lungs. Transmission electron microscopy further showed a significant amount of lamellar bodies in CeO$_2$ and CeO$_2$ plus DEP-exposed AM. At 28 days post-exposure, increased lung hydroxyproline content was evident in the CeO$_2$-exposed lung tissues. Histopathological analysis of CeO$_2$-exposed lungs showed large (alveolar sized) clumps of acellular material, but no granulomatous lesions at 28 days post-exposure. However, morphometric evidence, using Sirius red staining for collagen, demonstrated that rats exposed to the combination of CeO$_2$ plus DEP exhibited significantly greater fibrosis than CeO$_2$ or DEP alone. These results show that CeO$_2$ exposure induces M2 AM differentiation, apoptosis, OPN expression, and lung fibrosis.

**258 DETECTION OF NANOPARTICLES IN THE PERFUSATE OF FLOW-THROUGH SKIN DIFFUSION CELLS.**

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Little is known about TiO$_2$ and ZnO nanoparticles (NP) in UBV-damaged skin. In this study, weanling pigs were exposed to UBV (moderate erythema). The skin was dermoleated and placed in flow-through diffusion cells. The UV-radiated and the unexposed skin were treated with 4 sunscreen formulations: 10% rutile TiO$_2$ in oil/water (o/w) and water/oil; 5% coated ZnO o/w; and 5% uncoated ZnO o/w. TiO$_2$ had a particle size of 10x50 nm (agglomerates, ca. 40-60 nm). ZnO had a mean size of 140 nm (ca.60-200 nm). Skin was perfused for 24h and processed for various microscopic techniques and for inductively coupled plasma – mass spectrometry (ICP-MS). Perfusate was analyzed for Ti or Zn by ICP-MS. Ti or Zn was not detected in the perfusate samples independent of exposure, skin formulation, or UVB radiation. Additionally, the perfusate samples were concentrated and analyzed for elemental content and by transmission electron microscopy / Energy Dispersive X-Ray Spectroscopy (TEM/EDXS). Inorganic NP was detected in some of the perfusate samples of the UVB exposed and the unexposed skin. Unique spherical particles were identified by EDXS as SiO$_2$, nanoized NaCl crystals as well as Al, Mg, K and Ca containing NP. Spherical TiO$_2$ NP having a different crystal structure than the tested form (by Field Emission Gun-TEM and High-Angle Annular Dark-Field Scanning Transmission Electron Microscopy) were detected. These NP in the perfusate was independent of exposure or non-exposure. SAXS (Small angle X-ray scattering) also confirmed that there was no correlation between these TiO$_2$ NP and the TiO$_2$ used in the sunscreen formulations. These results show that NP are ubiquitous even under strictly controlled conditions and it is extremely important to use suitable analytical methods to ensure correct data assessments.

**259 LECTINS MODULATE MULTI-WALLED CARBON NANOTUBES CELLULAR UPTAKE IN HUMAN EPIDERMAL KERATINOCYTES.**

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The development of nanomaterials for biomedical applications has attracted a great deal of attention. Carbon nanotubes may interact and cross cell membranes and serve as potential carriers for drug delivery studies. The reflection mode in the confocal laser scanning microscope was used to image multi-wall carbon nanotubes (MWCNT) uptake in human neonatal epidermal keratinocytes (HEK) stained with the cytoskeleton protein F-actin. Scanning electron microscopy depicted tight binding of MWCNT on the plasma membrane of HEK while some MWCNT were located in the cell. Since keratinocytes normally engulf melanosomes, we hypothesized that the melanocyte transfer pathway could be a potential route of entry into keratinocytes. Lectins which are inhibitors of melanosome transfer were used to study the uptake by MWCNT in keratinocytes, to see if they played a role in reducing the cellular uptake of MWCNT in HEK. Three different lectins, *Pisum sativum* (PS), *Lycopersicon esculentum* (LE), and *Tetragonobulus purpureus* (TP) were used as a cocktail. The maximal concentrations of lectins that would be non-toxic to the HEK was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. These studies confirmed that lectin cocktails (PS 5μg/ml, LE 2.5μg/ml and TP 2.5μg/ml) decreased MWCNT interaction with the cell surface and uptake. F-actin, a cytoskeleton protein, was used to visualize how the MWCNT interacted with the keratinocytes in the cells. MWCNT were found to traverse through the cells’ cytoskeleton and the plasma membrane into adjacent cells. (Supported by Dermal Toxicology Specialty Section of the National Society of Toxicology Bartelle Student Research Award)

**260 TITANIUM DIOXIDE EXPOSURE INHIBITS ALLERGIC ASTHMA RESPONSE.**


Effects of airways exposure to different materials may depend on the allergic status of the exposed individuals and that we have investigated the pulmonary inflammatory responses against two different sized of titanium dioxides on the allergic asthma mouse model. Mice were allergen sensitized by administering ovalbumin-aluminum suspension intraperitoneally on day 1 and 10 and exposed either to nanosized or coarse TiO$_2$ aerosol (10 mg/m$^3$) for two hours on day 18-21 of the 22 day experiment. On days 19-21 the mice were given 50 micrograms of ovalbumin intranasally to elicit asthma. On day 22 the bronchial responsiveness to methacholine was determined by whole body plethysmography, after which the mice were sacrificed. Inflammatory cell infiltration was characterized from bronchoalveolar lavage fluid, and expression of cytokines and chemokines relevant to inflammation in the lung tissue was assessed by real-time PCR. Interestingly, asthmatic mice showed remarkable suppression of most mediators and signs of allergic asthma when exposed to both nanosized and coarse TiO$_2$. The levels of leukocytes, cytokines, chemokines and antibodies relevant in allergic asthma were lower in the lungs of mice exposed to TiO$_2$ particles compared to the naïve animals. However, in other cases, the differences were less marked. These results suggest that there is a systemic inhibition caused by titanium dioxide inhalation that prevents symptoms of allergic asthma. However, on the other hand it is of notice, that exposure can pose other dangers. It can be therefore concluded that in assessing health implications of particle exposure in human populations it is remarkably important to consider the heterogeneity of the state of health of individuals in that population.

**261 ACELLULAR ASSESSMENTS OF ENGINEERED-MANUFACTURED NANOPARTICLE BIOLOGICAL SURFACE REACTIVITY.**

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It is critical to assess the surface properties and reactivity of engineered-manufactured nanoparticles (NPs) as these will influence their interactions with biological systems, biokinetics and toxicity. We examined the physicochemical properties and surface reactivity of metal oxide NPs (TiO$_2$, CeO$_2$, and SiO$_2$) in several acellular assays to examine their “reactive” metal content, nitrosylation potential and ability to deplete nitric oxide (NO). The thiobarbituric acid substance assay (TBARS) was used to examine NP reactive metal content. Newly developed assays assessed NP nitrosylation and NO depletion properties. TBARS and nitrosylation assays were modified to determine photocatalytic properties of NPs. Nanoscale (<100 nm) NPs displayed the greatest activity compared to non-nanoscale (>100 nm) NPs in all assays where activity was detected. At equivalent concentrations SiO$_2$ and CeO$_2$ NPs displayed no endogenous or photocatalytic activity when examined in either the TBARS or nitrosylation assay. In contrast, at equivalent concentrations TiO$_2$ NPs displayed low levels of detectable endogenous and very high levels of photocatalytic activity in the TBARS assay. TiO$_2$ NP TBARS activity did not correlate with size or surface area as maximal activities were observed in the 25–32 nm size range and were influenced by crystallinity structure. NP displayed variable NO depletion potential (CeO$_2$>TiO$_2$>SiO$_2$). TiO$_2$ NO depletion did not correlate with size or surface area as maximal activities were observed in the 25–32 nm size range and were influenced by crystalline structure. In contrast CeO$_2$ NO depletion did correlate with size and surface area. These results demonstrate that the complex biologic effects of NP on oxidative stress, protein modification, cellular function and toxicity preclude a simple prediction based on size and surface area alone. (This abstract does not reflect EPA policy)

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**262 TITANIUM DIOXIDE NANOPARTICLES CAUSE GENOTOXICITY IN HUMAN LUNG EPITHELIAL CELLS.**


The use of engineered nanoparticles in consumer products is steadily increasing. The use of engineered nanoparticles in consumer products is steadily increasing. However, the health effects of exposure to these nanoparticles are not thoroughly understood. This study investigated the genotoxicity of six titanium dioxide and
two cerium oxide nanoparticles of various sizes and manufacturers. Immuno spin trapping (IST), an acellular assay, was used to determine DNA radical production. Based on an initial screening with IST, two titanium dioxide particles and one cerium oxide particle were selected for human retinal epithelial cells (BEAS-2B) cultured in KGM defined medium. Trypan blue dye exclusion was used to establish a dose-response curve of cell toxicity. Only Degussa P25 AEROXIDE® TiO2 was cytotoxic at the highest dose tested, 150 μg/ml after 24 hours of exposure. The alkali comet assay was used to assess DNA single-strand breaks in culture after 24 hr treatment with different concentrations of nanoparticles (10, 50, 100, 150 μg/ml). Degussa P25 AEROXIDE® TiO2 (27.5 nm) and Alfa Aesar TiO2 (32 nm) caused significant single strand breaks in DNA perhaps due to oxidative modification at concentrations of 100 and 150 μg/ml. Tail DNA was significantly elevated compared to control for Degussa P25 AEROXIDE® TiO2 and Alfa Aesar TiO2 (32 nm) at all concentrations after 24 hr treatment. The range of average particle size in suspension was significantly larger than the manufacturer’s listed size due to sub-staitional agglomeration in the liquid phase. The results suggest that titanium dioxide nanoparticles, Degussa P25 AEROXIDE® TiO2 and Alfa Aesar TiO2 (32 nm) (anatase and rutile mixtures) caused oxidative damage to DNA. Additionally, the results indicate that Degussa P25 AEROXIDE® TiO2 and Alfa Aesar TiO2 (32 nm) may be genotoxic, potentially due to free radical formation and oxidative stress mecha-nisms. [Abstract does not necessarily reflect the policies of the U.S. EPA.]

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**263 NANO TiO2: AN ASSESSMENT OF POTENTIAL PHOTOTOXICITY IN RETINAL PIGMENT EPITHELIAL CELLS IN VITRO.**

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Nanoparticles often have properties, such as photoactivity, that differ from those of their bulk counterparts. Phototoxic materials can become photocytotoxic by the generation of reactive oxygen species and free-radical oxidative damage to surrounding tissues. The retina is the only part of the central nervous system directly exposed to light, and the retinal pigment epithelium (RPE) is a site of photoactivity. A human-derived RPE cell line (ARPE-19) was exposed to nano-TiO2 and observed under a brief or an extended time course, and after exposure to visible light or UV radiation. TiO2 nanoparticles (rutile, 30-40 nm, and anatase, 32 nm) were suspended in DMEM/F12 with 10% FBS, isolated, and characterized by dynamic light scattering. Cells were plated on culture slides, treated with 15 μg/ml nano-TiO2, and observed under phase microscopy from 5 min to 5 days. Particles immediately began to settle, and by 3 days large agglomerates were seen inside cells, often in ring structures around the nucleus. In a longer study, cells were treated with 0, 10, or 30 μg/ml nano-TiO2 and photographed for up to 25 days. The ring structures were visible in TiO2 flask at 24 hours and persisted in viable cells for up to 25 days. Finally, cells were exposed to 0, 1, 5, 10, 25, and 50 μg/ml TiO2 (anatase) in DMEM/F12 with 10% FBS for 24 hrs. Cells were switched to HBSS and exposed to visible light (1 hr), or UV radiation (15 min), then returned to media containing either 10% or 2% FBS. The next day cytotoxicity was assessed by MTS and live/dead assays (calcine-AM/propidium iodide). In 2% FBS, cells exposed to 25 or 50 μg/ml TiO2 and visible light or UV radiation showed significantly lower MTS and lower % viability than dark controls. These experiments demonstrate that TiO2 nanoparticles can accumulate in ARPE-19 cells within hours, persist for weeks, and may become photocytotoxic when exposed to visible or UV wavelengths. This abstract does not reflect EPA policy.

**264 COMPARATIVE RESPONSE OF PRIMARY MOUSE HEPATOCYTES CULTURED FOR 1 DAY AND THREE WEEKS TO QUANTUM DOT EXPOSURE.**

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Quantum dots (Qdots) are semiconductor nanomaterials that are being promoted with much enthusiasm. Their unique photoluminescent properties make them ideally suited to many applications, including molecular and cellular research and biomedical applications. Two notable proposed uses in the biomedical field include diagnostic imaging and drug delivery. Because of their intended use as therapeutics and because of the anticipated occupational exposures, there is a great need for toxicity testing.

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**265 TRANSCRIPTIONAL AND CYTOTOXIC DIFFERENCES OF NANO-ZINC OXIDE AND NANO-TITANIUM DIOXIDE IN COLON AND SKIN-DERIVED CELL LINES.**

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Manufactured nanoparticles have unique properties due to their size, shape, composition, surface area, and chemical composition. These properties can be attractive for a broad range of novel applications but concern remains regarding the toxicity of these materials. Micronized zinc oxide (ZnO) and titanium dioxide (TiO2) are nanomaterials that have found utility in sunscreens and cosmetics for UV protection of exposed skin for aesthetic reasons since they are transparent rather than white in color. These materials are generally considered safe, including on intact skin since they do not penetrate past the stratum corneum layer into the proliferative or live skin cell layers. However, our previous work, and the work of others, demonstrates that commercial grade powders of nanoZnO have considerable cytotoxicity when in contact with various live human cells. We initiated studies using RKO (colon cancer) cells as we were concerned with potential ingestion toxicities of a range of nano-metal oxides, but we have also recently utilized Sk-Mel-28 (melanoma) and HaCaT (transformed keratinocytes) cells in our studies. While nanoZnO and nanoTiO2 agglomerate into large clusters in media, the agglomerates interact with the cells and we observe that nanoZnO is considerably more cytotoxic than nanoTiO2. In addition, all of these cell types display pronounced transcriptional responses within 4 hours of exposure to nanoZnO. A prominent response is consistent with metal ion responsiveness like metallothioneins and zinc efflux transporter induction. However, the transcriptional responses also show particulate-dependent induction of select genes associated with autophagy. Biochemical evaluation of autophagy also validates that these nanomaterials can promote autophagy. Therefore, a component of nanomaterial cytotoxicity may be the induction of autophagic responses.

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**266 IN VITRO GENOTOXICITY OF FIVE TITANIUM DIOXIDES.**

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Titanium dioxide (TiO2) nanoparticles are known to induce oxidative stress, inflammation, and cytotoxic effects in mammalian systems. The toxic mechanisms of nanosized TiO2 are not fully understood, and information on the genotoxicity of TiO2 is still limited. In our study, we assayed three types of commercial nano-TiO2 (nano-anatase, <25 nm; nano-rutile, 30-40 nm, with 10% anatase; silica-coated nano-rutile, 10x40 nm) for their genotoxicity. Two fine-sized forms of TiO2 (rutile, <5 μm; anatase, <170 nm) were studied for comparison. The Comet assay was used to assess DNA strand breaks (DNA damage) and the cytokinesis block micronucleus (CBMN) assay to detect chromosomal damage. DNA damage was examined in cultured human mesothelial (MET5A) and bronchial epithelial (BEAS 2B) cells treated with TiO2 for 24, 48 and 72 h. The induction of micronuclei was scored in BEAS 2B cells after 48- and 72-h treatments. Five doses (10-160 μg/cm²) of each TiO2 were studied in both assays. Positive and negative controls were included in all experiments. Our results indicated that nano-anatase, nano-rutile and silica-coated nano-rutile significantly induce DNA damage in MET5A cells at all treatment times. Similarly, fine-rutile and fine-anatase induced DNA damage in MET5A cells.
at all treatment times, except for fine-anatase after the 48-h treatment. In BEAS 2B cells, DNA damage was increased by nano-anatase after the 48-h and 72-h treatments and by nano-rutile and silica-coated nano-rutile after the 24-h and 72-h treatments. A positive result was also obtained with fine anatase at 24 h. In the CBMN assay, none of the TiO2 types induced micronuclei. In conclusion, our results indicate that all five TiO2 materials assayed increased DNA damage in human MTEAS cells but were less effective in human BEAS 2B cells. None of the TiO2 particle studied induced MN in BEAS 2B cells. [Funded by the European Commission (NANOSH, NMP4-CT-2006-032777)]

267 DIFFERENTIAL CYTOKINE RESPONSES INDUCED BY PLAIN AND RHODAMINE-MODIFIED SILICA-NANOPARTICLES IN EPITHELIAL LUNG CELLS.
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Sponsor: M. Lawl.

Nanoparticles (NPs) of amorphous silica particles are used in a large range of products. Inhalation of such NPs may induce inflammation, and may potentially represent a health hazard as too strong and persistent inflammation is considered as a key event in the development of lung disease. In this study we have investigated how modifying the particle surface may affect cellular responses. We have compared the potential of plain silica NPs (50 nm) and rhodamine-labelled silica NPs (50 nm) to induce cytokine responses in human bronchial epithelial lung cells (BEAS-2B) and in primary epithelial alveolar cells from rats. The relationship to differential activation of different signalling mechanisms (src, MAP-kinase, NFkB) and particle uptake in the cells was also examined (in BEAS-2B cells). The release of interleukin (IL)-6 and the chemokine CXCL18 (IL-8) was studied by ELISA, and the expression patterns of these cytokines were measured by real-time PCR. The importance of signalling mechanisms (p-src, p-38, p-JNK, P-ERK, p65, and IkBα) was studied by Western analysis, and by use of different chemical inhibitors. Particle uptake was studied by confocal microscopy. The results showed that the rhodamine-labelled silica NPs induced markedly stronger IL-6 and IL-8 responses than the unmodified NPs. Similarly, the Western analysis showed most marked responses to rhodamine-labelled NPs, and in particular for p-src and p-JNK. The cytokine responses were substantially reduced by inhibition of p38 and JNK, less by inhibition of src and not by inhibition of ERK. Confocal microscopy did not show any uptake of rhodamine-labelled NPs. In conclusion, these results show that modification of the silica surface with rhodamine strongly increase the cytokine responses in epithelial lung cells, and suggest that this is related to activation of the MAP-kinases, JNK and p38, and possibly to src.

268 OXIDATIVE STRESS OF AMORPHOUS MONODISPERSE SILICA NANOPARTICLES IN HUMAN ENDOTHELIAL CELLS.
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The aim of this study was to investigate if oxidative stress is apparent after sub-toxic dosing of silica nanoparticles (SNP) in human endothelial cells (EAHY926 cell line). Well characterized amorphous (monodisperse) spherical SNP with a diameter of 16 and 60nm were used. Endothelial cells were incubated with nanoparticles at the concentrations from 25 to 50μg/ml and the samples were collected at different time points. Cell membrane integrity was assessed by measuring extracellular lactate dehydrogenase. The concentration of malondialdehyde (MDA) and 4-hydroxynonenals (HNE) was determined as markers of lipid peroxidation. HPLC–MS method was used to quantify intracellular reduced (GSH) and oxidized glutathione (GSSG). Real-Time PCR was used to assess expression of inducible genes in response to oxidative stress at 40μg/ml SNP. The significant increase of MDA and HAE concentration was observed only in cells treated with hydrogen peroxide (positive control) compared to control cells (7.7±4.3 nM/g proteins vs 1.3±0.3 nM/g proteins, respectively). The significant increase in intracellular GSSG/GSH ratio was noticed after 1 and 4h exposure with hydrogen peroxide, and after 4h incubation with 50 μg/ml of 16nm SNP, however, already high mortality of cells was observed. Higher expression of oxidized low density lipoprotein (lectin-like) receptor 1 gene was measured in samples treated for 2, 6 and 24h with 16nm SNP whereas higher expression of heme oxygenase 1 gene was observed only after 6h treatment with 16nm SNP. The results suggest that oxidative stress is not the main mechanism contributing to cytotoxicity for the amorphous silica nanoparticles tested. Work was financed by the Belgian Ministry of Scientific Policy in the frame of S2NANO project (contract number SD/HE/02A).

269 HEPATIC GRANULOMATOUS FORMATION IN NANOCERIA INFUSED RATS, IMPLICATION FOR NANOPARTICLE SAFETY.
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Objectives: We have recently reported tissue biodistribution with a relative lack of injury in male rats after acute i.v. infusion of ceria nanoparticles. In this study we examine possible long-term effects of ceria on peripheral organs and brain. Methods: Five nm ceria nanoparticles with citrate surface coating were synthesized and fully characterized for i.v. infusion at 100 μg/kg dose in male Sprague Dawley rats. After a single infusion, rats were terminated 30 days later. Multiple organs were processed for LM and TEM analysis. Other endpoints included cerium assay and oxidative stress index. Results: Overt ceria accumulation in the brain was not observed. Enlargement of spleen was apparent. The general architecture of the liver was not altered; however, granulomatous formation in hepatic parenchyma appeared in the ceria-infused rats. Histology of the nodules consisted of Kupffer cells encircled by varying amounts of mononucleated cells. Masson trichrome stain indicated no excessive collagen accumulation. The internalized ceria nanoparticles in Kupffer cells appeared as spherical to oval agglomerates of varying dimensions by TEM. Smaller, more scattered ceria aggregates were also observed in cytoplasm of the hepatocytes with many ceria clusters appearing adjacent to bile canaliculi. The latter, however, rarely contained ceria nanoparticles. Hepatocyte density showed a slight decline in comparison to the saline-infused control. Conclusion: This first report of ceria nanoparticle-induced hepatic granulomatous formation coupled with the observed splenomegaly carries considerable implication for environmental health and human safety for this type of nanoparticle; particularly in light of its extensive industrial application including the use as fuel additive and its prospective replacement for zinc oxide and titanium oxide in sunscreens. [Supported in part by U.S. EPA STAR Grant RD-833772].

270 TOXIC EFFECTS OF METAL/METAL OXIDE NANOPARTICLES IN SKIN MODEL.
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Metal/metal oxide nanoparticles (Me/Mo NP), e.g. nickel (Ni), cobalt (Co), nickel oxide (NiO), and cobalt oxide (Co3O4), are commercially available and used by the medical/chemical industries for a number of pharmaceutical and engineering applications. The physical nature and reactive surface properties of NPs may affect their ability to induce dermal toxicity thus causing adverse skin reactions. Although the effects of Ni and Co on skin are well known (hypersensitivity, contact dermatitis and cancer), the dermal effects of Me/Mo NPs are unknown. We hypothesize that NPs may be toxic via the metal's ability to generate reactive oxygen species, initiate oxidative stress, and induce redox-sensitive transcription factors thereby affecting/leading to inflammation. Due to the skin's susceptibility to UV radiation, it is important to address the combined effect of UVB and NPs. To test the hypothesis, the effects of Me/Mo NPs (Co and Ni) were studied in vitro and in situ using murine epidermal cells (JB6 P+/-) and an engineered human skin (EpiDerm FT). Exposure of JB6 P+/- cells to NPs resulted in the generation of hydroxyl radicals and activation of AP-1 and NF-κB. Co-exposure of JB6 P+/- cells to UVB and NPs significantly accelerated accumulation of oxidative stress products, induced release of cytokines, cell damage and death. Co-exposure of engineered skin to NPs and UVB caused epidermal thickening, activation of dermal fibroblasts, accumulation of protein carbonyls, and pro-inflammatory cytokines. Altogether, these data indicate that co-exposure of dermal cells and engineered skin to UVB and Me/Mo NPs was associated with oxidative stress, and release of inflammatory cytokines is comparable to those treated with NPs alone. Therefore, it is imperative to assess the adverse effects of UVB when evaluating dermal toxicity of engineered NPs on skin.

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MANGANESE NANOPARTICLE CHARACTERIZATION AND POSSIBLE NEUROTOXIC MECHANISMS IN A DOPAMINERGIC NEURONAL MODEL.

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Chronic exposure to manganese (Mn) has been implicated in the pathogenesis of Parkinson’s disease (PD). MAN-made production of nanoparticles is burgeoning, increasing the probability of exposure in occupational settings. The related issue of nanoparticle toxicity has led to investigations evaluating effects of metal nanoparticles like Mn. Unfortunately, in vitro nanoparticle toxicity studies are limited by still unresolved problems relating to agglomeration and controlled dosing of nanoparticles. In this study, we systematically characterized Mn-nanoparticle size for use in N27 dopaminergic neuronal cells and then examined the metal nanoparticle-induced oxidative signaling. A combination of newly developed Differential Interference Contrast (DIC) microscopy and Transmission electron microscopy (TEM) techniques demonstrated that the Mn nanoparticles agglomerate in 10% serum RPMI media, ranging in size from single nanoparticles as small as ~25 nm to agglomerates of up to ~900 nm. Additional DIC studies showed that both fine- and nano-sized WC-Co induce ROS generation, cell proliferation, and activation of specific cell signaling pathways. These studies also underscore that size is a critical factor in assessment of toxicological and biological responses of WC-Co materials.

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PULMONARY TOXICITY OF INSTILLED METAL NANOPARTICLES IN THE RAT.

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As the use of nanoparticles has become mainstream, a fundamental understanding of the underlying toxicity of these materials is important as occupational and operational exposures become more likely. This study was designed to assess the basic pulmonary toxicity of potentially relevant brass and aluminum nanolake particles. Suspensions of test nanoparticles, 20–40 nm thickness and 3–5 μm in major dimension, were intratracheally instilled into the lungs of male Sprague-Dawley rats. Nanoparticles were administered in three dose groups of eight rats: 0.1 mg/kg, 1.0 mg/kg, and 5.0 mg/kg. At 24 hours and 14 days post-exposure, the rats underwent necropsy procedures involving the collection of bronchoalveolar lavage fluid (BALF) samples and fixing of the whole lung for histopathological analysis. Histopathological analysis reveals, at the 0.1 mg/kg dose, brass nanoparticle exposure results in edema by 24 hours post exposure and the development of fibrosis by 14 days post exposure. On the other hand, exposure to aluminum nanoparticles at 1.0 mg/kg does not produce any signs of acute injury. However, macrophages are highly pigmented indicating uptake of the aluminum nanoparticles. Interleukin-1 beta (IL-1β) concentrations in the BALF of all three groups was monitored as an indicator of inflammation. At 24 hours post exposure IL-1β levels in the brass 1.0 and 5.0 mg/kg groups were elevated (397 pg/mL and 233 pg/mL respectively) with respect to the 0.1 mg/kg and saline groups (25 pg/mL and 2.3 pg/mL respectively). By 14 days post exposure IL-1β levels in all four brass groups had returned to less than 25 pg/mL. At 24 hours post exposure IL-1β levels in the saline, 0.1, and 1.0 mg/kg aluminum groups were all below 10 pg/mL. The 5.0 mg/kg aluminum group was elevated to 112 pg/mL. By 14 days post exposure IL-1β levels were slightly elevated in the 1.0 and 5.0 mg/kg groups (48 pg/mL and 83 pg/mL respectively). These data support the theory that brass nanoparticles would cause acute lung toxicity whereas aluminum would produce a slower onset but longer lasting inflammatory response.

CELLULAR RECOGNITION AND TRAFFICKING OF ANIONIC NANOPARTICLES BY MACROPHAGE SCAVENGER RECEPTOR A.

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The internalization of nanoparticles (NPs) into cells is known to involve active transport mechanisms, but the precise biological molecules involved in these processes are poorly understood. We demonstrate that the level of uptake of anionic polystyrene and amorphous silica NPs (20–200 nm diameter) in a macrophage cell line is strongly inhibited by silencing expression of endogenous scavenger receptor A (SR-A), whereas NP uptake is significantly enhanced by introducing SR-A into human cells that are normally non-phagocytic. SR-A dependent NP uptake was observed both in the presence or absence of serum proteins, suggesting recognition of
NP uptake by SR-A involves more than simple anionic charge interactions. High resolution<br>fluorescence microscopy analyses show that the majority of single or small clusters of silica NP's co-localize and traffic intracellularly with SR-A and are internalized through a pathway characteristic of clathrin-dependent endocytosis. In contrast, larger agglomerates (>500 nm diameter) of the same silica NPs show only a low level of co-localization with the receptor, suggesting independent pathways for internalization are involved. Silencing of SR-A expression also results in decreased NP-induced secretion of pro-inflammatory cytokines, suggesting the inflammatory response is triggered by NP internalization rather than by non-specific cell contact. Given the broad expression of this receptor throughout the reticuloendothelial system, the SR-A pathway likely plays an important role in modulating inflammatory responses to NPs and in limiting the effective delivery of therapeutic NPs in vivo.

276 EVALUATION OF TOPO-PMAT MODIFIED QUANTUM DOT UPTAKE AND TOXICITY IN A549 HUMAN LUNG EPITHELIAL CELLS.
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Nanotechnology is becoming increasingly more prevalent in modern society. The ability to engineer various forms of nanoparticles has led to their use in products such as cosmetics and LED displays, and even biomedical imaging. Quantum dots (QDs) are one form of fluorescent nanoparticles. However, their heavy metal composition has given rise to concerns regarding toxicity and persistence in biological settings. The aim of this research is to evaluate the in vitro toxicity of triocystophosphine oxide, poly(maleic anhydride-alt-1-tetradecene (TOPO-PMAT) coated CdSe QDs on human lung carcinoma epithelial A549 cells. QD uptake was measured following a 24 hour exposure with doses from 2.5 nM to 40 nM, using both flow cytometry (FACS) and fluorescence microscopy, which demonstrated a dose-dependent increase. Cell viability was unaffected at these doses, as assessed by MTT reduction to formazan. However, Western blot analysis of heme oxygenase 1 (HOX) levels showed a dose-dependent up-regulation, suggestive of oxidative stress. Total glutathione (GSH) and total cellular thiols were assessed using NDA and monobromobimane (MBB) fluorescence, respectively. Neither of these measures changed. The induction of HMOX is thus more suggestive of an inflammatory response. While these preliminary results suggest that these amphiophilic QDs are taken up and do not have an adverse affect on cell viability, exposure does cause a stress response in A549 cells. This work was supported by NEIHS grants R01ES016189.

277 SHORT- AND LONG-TERM BIODISTRIBUTION AND OXIDATIVE STRESS EFFECTS OF A SYSTEMICALLY-INTRODUCED 5NM CERIA ENGINEERED NANOMATERIAL.
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Objective: To characterize the short- and long-term biodistribution and persistence of a 5 nm diameter ceria dispersion from blood and its oxidative stress effects in the spleen. Methods: An - 4% aqueous citrate-stabilized ceria dispersion, synthesized and characterized in-house, was intravenously infused into rats (100 mg/kg), which were terminated 1 or 20 h or 30 days later. Ceria concentration and localization in the brain, liver, spleen and whole blood were assessed by ICP-MS and light and electron microscopy. Oxidative stress effects were assessed as protein bound 4-hydroxy-2-trans-nonenal (HNE), 3-nitrotyrosine (3NT), and protein carbonyls (PC). Results: Cerium was quantified in cortex samples 1 and 20 h and 30 d after ceria dosing. However, EM revealed the presence of ceria aggregates in the brain vascular compartment but not in microvascular endothelial or brain cells. Thirty days after the ceria infusion the cerium concentration in the liver and spleen were 60 and 35% of that seen 20 h after infusion. However, there was no significant difference between total cerium in liver and spleen 30 d vs. 20 h after ceria infusion because the liver and spleen weights of treated rats were greater than control rats. At 30 d 44% and 10% of the ceria dose was in the liver and spleen. Giant cells containing ceria were seen in spleen red pulp as well as thickened arteriels in white pulp. LM and EM revealed granulomatous formations in the liver. Oxidative biomarkers in spleen revealed 10% elevation of protein oxidation and 10% decreased lipid peroxidation. Conclusions: Contrary to expectation, these small ENMs did not permeate the BBB. Ceria clearance from these organs was slow. More toxicity was seen in the spleen and liver after 30 days than at 1 or 20h, demonstrating the importance of studying long-term retention and effects of engineered nanomaterials. Supported by U.S. EPA STAR Grant RD-833772.

278 EFFECTS OF PARTICLE SIZE AND ROUTE OF EXPOSURE ON THE BIOAVAILABILITY OF ZINC FROM NANO-SIZED ZINC OXIDE PARTICLES.
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This study utilized nano-sized ZnO to better define the pharmacokinetic and translocation properties of nano-sized particles. To determine the pharmacokinetic and translocation properties of neutron activated Zn (65Zn) particles, we utilized two particle sizes (7-13 nm and 40-100 nm) and two routes of exposure (intra-venous injection (IV) or intratracheal instillation (IT)). Male Sprague-Dawley rats were given a single dose of neutron activated 65ZnO particles at 1.5 mg/kg body weight. At varying time points, tissues (lungs, brain, heart, spleen, kidney, skeletal muscle, liver, bone marrow and gastrointestinal tract) were collected. The radioactivity in tissues was calculated as % of instilled/injected dose. Results show that at 30 minutes post-IV, the % of injected 65Zn in the liver was significantly higher for 40-100 nm than for 7-13 nm. However, 7 days later, liver uptake was the same for both particle sizes. For the IT-instilled particles, at both 1 and 7 days post-IT, tissue distribution was similar between the 2 particle sizes. At 1 day post, only 10% of the instilled 65ZnO remained in the lung for both particle sizes. At 7 days post, the amount of 65ZnO in the lung decreased to 0.1% for both particle sizes. In conclusion, for the IV-instilled particles, particle size has an initial effect on 65Zn uptake in the liver. For IT instilled particles, particle size did not alter lung clearance or distribution. The lack of particle size dependency on translocation and lung clearance of 65ZnO particles may be due to the agglomerated state of the ZnO nanoparticles. Dynamic light scattering revealed that the 7-13 nm and 40-100 nm ZnO particles had a mean diameter of 83.5 nm and 88.3 respectively. Also, the high solubility of ZnO could have obscured any influence of particle size on pulmonary deposition of the particles. The 65Zn measured in collected tissues was most certainly dissolved 65Zn from the particles, especially at late time points.

279 AGGLOMERATION STATUS OF NANO- AND SUBMICRON-SIZED PARTICLES AND THE EFFECT ON PULMONARY TOXICITY.
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Nanoparticle (NP) toxicity testing comes with many challenges. One of them is agglomeration of nanoparticles in physiological media. In this study, we address the effect of agglomerated versus single particle suspensions of nanosized and submicron-sized gold on inflammatory response in the lung. Colloidal gold was chosen as a model particle to study effects on lung inflammatory markers in bronchoalveolar lavage fluid (BALF) after intratracheal instillation in the rat. A single dose of 1 mg of spherical gold particles of 50 nm or 250 nm is diluted 10% either by ultrapure water or by adding 10x phosphate buffered saline (PBS). Particles diluted in ultrapure water are well dispersed, while the citrate shell is disturbed and agglomerates are formed when diluting in PBS. A single dose of 1 mg DQ12 quartz is used as a positive control. Dynamic light scattering (NanoSight) is used to determine the particle size distribution in the suspensions prior to application. Cell differentials, oxidative stress and inflammation are measured in BALF after 24 hrs. This study focuses on the preparation of particle suspensions, the agglomeration status of particles and the effect this has on pulmonary inflammation.

280 THE PARTICOKINETIC AND PHYSIOLOGICAL BASIS FOR IN VITRO - IN VIVO EXTRAPOLATION OF NANOMATERIAL TOXICITY STUDIES.
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The rapid development, varied forms and potentially vast number of untested nanomaterials pose a significant challenge to time consuming and costly conventional safety assessment paradigms. This challenge will be met with new testing par-
Adipocytes such as Tox21™ and Toxcast™, which will utilize high throughput cell-based assays, limited animal data, informatics and computational tools for prediction and use of physiologically based pharmacokinetic models for extrapolation (NRC 2007). Approaches to relate cellular dose of nanomaterials from in vitro tests (15 to cellular and target tissue specific doses in humans and rodents exposed to nanomaterials is required to support both adequate design and robust interpretation in vitro studies of nanomaterials. We have developed in vitro and in vivo particokinetic models for nanomaterials which capture their unique kinetics in these systems. These models are used to establish and articulate the particokinetic and physiologival basis for in vitro-in vivo extrapolation of nanotoxicity studies. We demonstrate that in vitro, the most commonly used metric of dose, nominal media concentration, is, under most conditions, not suitable for dose-response or for extrapolation to in vivo studies because ignoring the effects of gravity and diffusion on delivery to cells introduces errors in dosimetry on the order of magnitude. Correcting for these errors and applying models of pulmonary nanoparticle deposition and systemic disposition, we show how computational particokinetic tools can be used to extrapolate tissue and cellular dose across the in vitro/in vivo divide based on particle surface area, or particle number as the metrics of dose. Conversely, we show how the same tools can be used to design in vitro nanomaterial toxicity studies which utilize doses which correspond to potential human exposures at OSHA permissible exposure levels for common particulates as a proxy for potential human exposure to nanomaterials.

**THE ROLE OF BRAIN MICROVESSEL ENDOTHELIAL CELLS IN THE NEUROTOXICITY OF SILVER OR GOLD NANOPARTICLES.**


The purpose of the current studies was to determine what role microvessel endothelial cells, which functionally comprise the blood-brain barrier (BBB), have in causing brain pro-inflammation state and subsequent neurotoxicity associated with exposures to colloidal metallic nanoparticles (NPs) like silver (Ag) and gold (Au). Our in vitro model of the BBB, a primary culture of rat brain microvessel endothelial cells (rMVEC), was isolated by a series of enzymatic digestions and differential centrifugation steps. Confluent rMVEC monolayers (10-14 days) were treated with various sized Ag (25, 40 or 80 nm) or Au (1.9, 3, 5, 7, 10, 30 or 60 nm) NPs. The cellular accumulation of the NPs was determined spectrophotometrically at various time intervals (30, 60 or 90 mins). The cytotoxicity was evaluated by cell proliferation assay (XTT) in rMVEC during a 24hr exposure to NPs (0.7 to 50 ug/ml). The influence of primary particle size (15 nm, 80 nm, 3 nm) as well as agglomerate size on nanoparticle endocytosis is exam-

**INTERNALIZATION OF SiO2 NANOPARTICLES: THE INFLUENCE OF SIZE ON METAL OXIDE NANOPARTICLE ENDOCYTOSIS.**

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The use of metal oxide nanoparticles in a variety of industrial, household, and medical products (e.g., cosmetics, diagnostics,itics, and insulators) is incr-

**BILOGICAL SURFACE ACTIVITY INDEX: A NOVEL METRIC TO CHARACTERIZE NANOMATERIAL INTERACTIONS IN BIOLOGICAL SYSTEMS.**

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The behaviors of nanomaterials in biological systems are dictated by the absorbed surface species. Quantitative assessment of the adsorption properties of the nano-

**COMPARATIVE TOXICOLOGICAL ANALYSIS OF QUANTUM DOTS AND WIRES ON HUMAN SKIN TISSUE.**

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Semiconductor quantum dots (QD) and wires are of growing technological rele-

**281 THE ROLE OF BRAIN MICROVESSEL ENDOTHELIAL CELLS IN THE NEUROTOXICITY OF SILVER OR GOLD NANOPARTICLES.**
WMCNT: The two main BSAL values of the nanomaterials were 3.11, -0.15, 0.72, 0.12, 0.64, 3.94, 0.98, 0.20, 0.45, 3.26, 4.18 and 3.17 for hydrophobicity; and -2.82, 0.86, -0.56, -1.73, -0.43, -4.78, -0.81, -1.24, -3.07, -0.87, -2.77 and -1.38 for hydrogen-bond basicity, respectively. These quantitative nano-descriptors can be used for predictive model development for biological activity and risk assessment of the nanomaterials (Supported by U.S. EPA STAR Grant # R833328 and US-AFOSR Grant # FAF550-08-1-0182).

285 UNDERSTANDING THE EFFECTS OF MICROCAPSULATION AND TEMPORAL INTRACELLULAR RESPONSE.
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Investigating the fate and behavior of quantum dots (QDs) within the body over time has become increasingly important due to the realization of their potential benefits in diagnostics and theranostics. Studies, in our and other labs, are currently underway to assess the efficacy of these nanomaterials as particles and in capsules for in situ tracking devices. It is imperative that QD toxicity is known prior to the utilization of these novel nanotechnological advances. Knowledge of QD-cellular temporal responses will guide further research. We have encapsulated cadmium selenide (CdSe)/zinc sulfide (ZnS) with negatively and neutrally charged hydrophilic polymers. Examination of these systems was carried out at biologically relevant pH and temperature to assess differences in the cellular reaction of human dermal fibroblasts. Cytotoxicity, mitochondrial activity, and protein up-regulation in cells exposed to QD materials were measured. Confocal microscopy was used to visualize the cellular morphology and trafficking of the QDs. Data was collected over a 48 hour time-course and 5 μM dose-response. Results show that cytotoxicity, mitochondrial disruption, inflammatory protein regulation, and adverse cellular morphology increase over time after QD and negatively charged QD-loaded capsules. It was noted that both pristine QD exposure concentrations produced similar toxicities, where compared to each other. The damaging effects of the QD materials seem to be mitigated when the particles are encapsulated; however, cells take up the negatively-charged capsules via an invagination mechanism. While the neutrally charged encapsulated QDs do associate with cells, uptake is not apparent as with the alternate material. Successful development of this system will lead to a greater understanding of the nano-bio interface and aid in the successful development of nanoparticle use in medical applications.

286 NICKEL NANOPARTICLES SYNERGISTICALLY ENHANCE PDGF-INDUCED CHEMOKINE PRODUCTION BY RAT PLEURAL MEOSITHELIAL CELLS.
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Nickel nanoparticles (NiNPs) are used as catalysts for the manufacture of multi-walled carbon nanotubes (MWCNT). We recently reported that inhalation exposure of mice to MWCNT caused subpleural fibrosis and aggregation of monocytes and lymphocytes at the pleural surface. Moreover, levels of platelet-derived growth factor (PDGF) were increased in the lungs of mice and rats exposed to MWCNT. We hypothesized that NiNPs or MWCNT act coordinately with PDGF to increase CCL2 and CXCL10 mRNAs. These data suggest that PDGF is an important regulator of nanoparticle-mediated immune responses at the pleural surface. (Fundled by NIHES R21-E5015801-01 and NIEHS RC2-E5018772-01).

287 HUMAN ENDOTHELIAL CELLS DISPLAY INFLAMMATORY MARKERS TO CACHE VALLEY PARTICULATE POLLUTION.
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Surrounded by mountains, Cache Valley is prone to strong wind inversions arising from motor vehicle emissions, and ammonia gas from animal excreta. These inversions caused the highest PM2.5 levels in the nation. Epidemiological studies from diverse geographic areas have linked air pollution exposures to particulate air pollution with stroke and Alzheimer’s disease and to early mortality from cancer and cardiovascular diseases, but studies to determine causal relationships between exposure to Cache Valley Particulate Matter (CVPM) and poor health outcomes have not been reported. To determine potential adverse consequences of exposures to CVPM on respiratory and cardiovascular organs, human bronchial epithelial cells (BEAS-2B) and human umbilical vein endothelial cells (HUVEC) were cultured with CVPM that was collected from various locations in the Cache Valley. CVPM was only slightly cytotoxic in BEAS-2B (<1,000 μg/ml), while HUVEC were substantially more sensitive to CVPM. CVPM at concentrations of up to 100 μg/ml produced a 2.5-fold increase in expression of IL-6 and a moderate increase in TNF-α. CVPM is a potent inducer of inflammation, causing a 2-fold increase in CRP production.

288 ZNO NANOPARTICLES ALTER MUCOSARIC LIGAND RECEPTOR LIGAND BINDING AND ACTIVATION OF STORE OPERATED CALCIUM ENTRY IN CHO CELLS.
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The ability of ZnO nanoparticles (20nm) to alter mucosaric receptor function was studied in CHO cells stably transfected with the gene for human M3 muscarinic acetylcholine receptor. Cytotoxicity was determined using the MTS assay. ZnO caused cytotoxicity in CHO cells in a concentration and time-dependent manner: Little toxicity was seen after a 3 hour exposure to ZnO at concentrations of up to 100 μg/ml, while half of the cells were killed following 24 and 48 hours exposure to ZnO concentrations of 40 and 25 μg/ml, respectively. Thus, CHO cells are relatively resistant to ZnO-mediated cytotoxicity. ZnO exposure had complex effects on receptor ligand binding functions: ZnO (3 h 20 μg/ml) increased the level of binding without effect receptor affinity for an antagonist probe ([3H]N-methylscopolamine; KD = 0.25nM). This may reflect a disruption of cellular metabolism. Agonist binding is sensitive to receptor interactions with transducer G proteins. ZnO NP eliminated high affinity agonist binding. ZnO also appeared to have an allosteric effect on ligand binding, slowing ligand dissociation at subtoxic concentrations. Short term (3.5h) exposure to ZnO decreased the initial phase of muscarinic receptor-mediated Ca2+ entry slightly, but virtually eliminated the sustained entry phase. Receptor activation releases IP3 from the membrane that then acts on receptors on the endoplasmic reticulum to release calcium. This depletion of ER calcium stimulates the entry of calcium from extracellular sources (i.e., store operated calcium entry; SOCE). SOCE were examined by acute exposure to thapsigargin (2μM) in a calcium-free medium, followed by addition of external calcium. Again, SOCE was severely depressed following exposure to the ZnO. These findings indicate that ZnO nanoparticles affect cellular signaling processes at both the receptor ligand recognition and signal pathway levels. These effects may have important implications for the use of these materials.

289 MALE REPRODUCTIVE TOXICITY STUDY OF QUANTUM DOT NANOPARTICLES IN MICE.
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Quantum Dots (QDs) are engineered semiconductor nanocrystals and currently applied in electronics industries, biomedical imaging, and drug targeting. A pilot study was performed to evaluate the potential toxicity of CdSeTe/ZnS (core/shell)
QDs with emission maximum at 800nm on the male reproductive system. C57BL/6j mice were intravenously injected via tail vein with 45pmol or 160pmol QDs per animal, and sacrificed at 12 hours or 28 days post injection, respectively. Whole body imaging demonstrated a rapid increase in fluorescence, primarily in the liver area, immediately following injection of QDs, followed by gradual decrease to baseline levels at 28 days post-injection in the mice treated with 45 pmol QDs whereas persistent fluorescence, still primarily in the liver area, even after 28 days in the mice treated with 160 pmol QDs. At 12 hours post-injection, both seminal vesicle weight and sperm head count were reduced in mice treated with ether dose. At this time point, there was also a decrease in testis weight of mice treated with 160 pmol QDs. However, no such alterations were observed in mice sacrificed at 28 days, suggesting the effects were transient or recoverable. Histopathological examination and TUNEL analysis of testis did not show significant QDs-associated changes. Persistence of QDs within body and the transient effects on the male reproductive system indicate that the reproductive toxicity of QDs warrants further study.

**290 INFLAMMATORY RESPONSES OF TITANIUM DIOXIDE WITH DIFFERENT SIZE AND PROPERTIES.**

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Nanotechnology has produced diverse nanomaterials such as nanosilica and titanium dioxide (TiO2). TiO2 is widely used as white pigments, in cosmetic applications or photocatalysis in air and water cleaning. However, TiO2 has recently been reported to induce pulmonary emphysema and its mechanism is not fully understood. In this study, we compared the inflammatory responses of rutile TiO2 with different size using human monocyte-like THP-1 cells. At first, we investigated the effects of TiO2 on the production of inflammatory cytokines (IL-1β, TNF-α and IL-8) by ELISA. Microsized-TiO2 induced the significant higher level of IL-1β, TNF-α and IL-8 production than non-treated cells, although nanosized-TiO2 did not induce the production of these cytokines. Similar results were observed in PMA-primed macrophage-like THP-1 cells. These results indicate that micro-sized-TiO2 would induce severe inflammation in vivo. Next we examined the mechanism of IL-1β production by micro-sized-TiO2, because IL-1β was reported to play a key role in the initiation of inflammation by TiO2. Mature IL-1β is produced by cleavage of the inactive pro-IL-1β by caspase-1. We showed that micro-sized-TiO2 induced the activation of caspase-1 using the caspase-1 inhibitor zYVAD-fmk. In addition, inhibiting actin with cytochalasin D inhibited the production of IL-1β, suggesting that phagocytosis is required for the activation of caspase-1 and micro-sized-TiO2. Because some particles are reported to induce the production of IL-1β through the Nalp3 inflammasome, we are now examining whether production of IL-1β by TiO2 would also occur through it.

**291 TUNABLE SUPERPARAMAGNETIC FE3O4-SIO2 CORE-SHELL NANOPARTICLES: SYNTHESIS, CHARACTERIZATION, AND IN VITRO COMPATIBILITY WITH IMMUNE-COMPETENT CELLS.**

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Nanoparticles are being considered for use in a wide variety of biomedical applications ranging from enhancement of image contrast in magnetic resonance imaging (MRI) to drug delivery systems. However, biocompatibility must be addressed in order to promote the safe use of nanomaterials. Silica is one of the preferred materials for surface coating when high biocompatibility, material stability and increase in residence time are desired. We report on a novel synthesis, by microemulsion technique, of monodispersed core-shell Fe3O4-SiO2 nanoparticles with a core size of 15 nm and an overall diameter of 25, 50 or 100 nm. Magnetic measurements were conducted to evaluate the performance of the core-shell nanoparticles as MRI contrast agents. Moreover, the biocompatibility of the tuneable, silica-coated core-shell nanoparticles versus commercially available dextran-coated iron oxide nanoparticles was assessed using primary human monocyte-derived macrophages and dendritic cells (DC). Cytotoxicity was evaluated by MTT and LDH release assay, and cytokine secretion was monitored by ELISA. In addition, cell internalization of nanoparticles was monitored using TEM and ICP-MS. Overall, due to their excellent magnetic properties, apparent low toxicity, stable dispersion at physiological pH, and the potential for further tailoring of particle properties through surface modifications of silica these core-shell particles are promising materials for drug delivery, magnetic cell separation, medical imaging, and other biomedical applications.

**292 COMPARATIVE TOXICITY OF SHORT-TERM AND CHRONIC EXPOSURE TO LUNAR DUST AND ITS COMPONENT PARTICLES IN HUMAN SKIN FIBROBLAST CELLS.**

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NASA is planning to construct a permanent space station on the moon by 2020. Humans will occupy this space station, therefore it is important to assess any health hazards present in the lunar environment. On prior missions to the moon, the dust that composes the lunar surface caused problems when it became stuck to the astronaut’s suit and contaminated the habitation module; NASA is concerned that this dust may be toxic to humans. This dust is composed of titanium, chromium, and silica. We obtained lunar dust simulates from NASA, in two different particle sizes; fine and very fine (JSC-1AF, JSC-1AVF respectively). In our study, we used human skin fibroblast cells as a model to investigate the cytotoxicity and genotoxicity of lunar dust and its component particles. We exposed the cells to simulated component concentrations ranging from 10-400 µg/cm2 for a treatment time of 24 and 120 h. After 24 h and 120 h JSC-1AF was more toxic than JSC-1AF, however JSC-1AF was less toxic after 120 h compared to 24 h. This trend was also displayed by silica which was the least toxic of the component particles and was less toxic after 120 h compared to 24 h. Chromium oxide was the most toxic of the component particles, followed by titanium dioxide, with silica being the least toxic. Our data for the 120 h genotoxicity show no chromosome damage induced by lunar dust and its component particles. This work is supported by the Maine Center for Toxicology and Environmental Health, NASA grant EP-08-01, and the Maine Space Grant Consortium.

**293 A FUNCTIONAL, REGULATORY ROLE OF INTESTINAL GAMMADELTA T CELLS DURING ESTABLISHMENT OF ALLERGIC SENSITIZATION.**

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Introduction: Food allergy affects approximately 5 % of all children and is the leading cause of hospitalization for anaphylactic reactions in westernized countries. It has been suggested that intestinal γδ T cells are of importance in the induction of oral tolerance. We therefore investigated whether establishment of food allergy induced changes in γδ T cells, and whether γδ T cells had functional relevance in allergic sensitization.

Methods: Mice were exposed to peanut and cholera toxin and changes in γδ T cells were measured. The UC7 anti-γδ TCR antibody was used to block the γδ TCR in vivo, after which mice were sensitized to peanut by using cholera toxin as mucosal adjuvant. After 4 weeks, peanut-specific antibodies in serum and cytokine production in spleen were measured.

Results: Induction of food allergy resulted in a profound decrease in the percentage of γδ T cells of importance in the induction of oral tolerance. We therefore investigated whether establishment of food allergy induced changes in γδ T cells, and whether γδ T cells had functional relevance in allergic sensitization.

Conclusion: These results demonstrate a unique regulatory role for intestinal γδ T cells during establishment of allergic sensitization.
Introduction: Diclofenac and other non-steroidal anti-inflammatory drugs (NSAIDs) are used as anti-inflammatory drugs. They interfere with the cyclooxygenase-mediated synthesis of prostaglandins, which are involved in immune responses. It is also known that NSAIDs are able to induce gastrointestinal damage. It was therefore of interest to investigate whether NSAIDs are able to enhance sensitization or abrogate tolerance to food antigens.

Methods: Mice were exposed to 0, 1, 10 or 25 mg/kg diclofenac and sensitized to peanut by using cholera toxin as mucosal adjuvant. In a tolerance model, oral tolerance was induced via feeding of peanut three weeks before sensitization with peanut. Diclofenac (1 mg/kg, oral gavage) was administered prior to peanut feeding. After 4 weeks, peanut-specific antibodies in serum and cytokine production in spleen were measured. Induction of intestinal damage 2 h after oral exposure with diclofenac was examined microscopically.

Results: Diclofenac-exposed animals showed increased levels of peanut-specific IgG1, IgG2a and IgE in serum compared to vehicle-treated animals. Furthermore, peanut-induced cytokine production in spleen was elevated upon diclofenac treatment. Importantly, diclofenac did not induce peanut-allergic responses in the absence of cholera toxin. In addition, intestines of diclofenac-treated mice did not show signs of tissue damage. Diclofenac was not able to disturb oral tolerance induction as peanut-specific IgG1 and IgE were still suppressed in peanut-tolerized mice compared to non-tolerized mice. On the other hand, peanut-specific T cell responses were significantly higher in diclofenac-treated tolerized mice compared to vehicle treated mice.

Conclusions: These data point towards an increased risk for induction of food allergy by diclofenac, when other circumstances are also in favor of induction of allergy.
Cleveland, while others were found more universally (Group 2). Asthma exacerbations were lower in remitted homes compared to non-remitted homes. The study objective was to compare the allergy induction potential of these groups of molds to户 whose mice with those using CBA/c mice. NICEATM evaluated 108 independent studies representing 15 substances in four vehicles in which 86 studies used CBA mice and 22 used BALB/c mice. Thirteen of these substances had guinea pig reference data and 12 had human reference data. LLNA outcomes using BALB/c are in agreement with LLNA outcomes obtained with CBA for 87% (13/15) of the test substances. LLNA outcomes with CBA agree with guinea pig outcomes for 92% (12/13) of the test substances and with human outcomes for 92% (11/12) of the test substances. LLNA outcomes with BALB/c agree with guinea pig outcomes for 77% (10/13) of the test substances and with human outcomes for 75% (9/12) of the test substances. A correlation analysis of log-transformed EC5 values calculated using LLNA data from each of the two strains indicates that the results from the two strains were correlated (r = 0.75). Overall, these data indicate that LLNA outcomes do not differ appreciably when either CBA or BALB/c mice are used as test animals. ILS staff was supported by NIEHS contract N01-ES-35594.

We have developed the h-CLAT, an in vitro skin sensitization test using THP-1 cells (human monocyte leukemia cell line). This test is based on the augmentation of CD86 and CD54 expression in THP-1 cells following exposure to chemicals. In this study the utility of h-CLAT to classify the skin sensitization potential by using various calculated values. From the data of 66 chemicals, which was both positive in h-CLAT and LLNA, several values were calculated as follow: 1) CV75, 2) maximum RFI of each chemical, 3) EC150, and 4) EC200. Correlational analyses between LLNA and the four values were performed. A statistically significant correlation was observed between CV75, EC150, and EC200 values with LLNA EC3. The EC150 value showed the better correlation compared to other values. From EC150 and EC200, Minimum Induction Threshold (MIT) was determined as a minimum value, smallest of either EC150 or EC200. MIT also show the good correlation with EC3. From these data, the h-CLAT might be a one of the useful tool to predict the allergic potency of chemicals after improving the detailed conditions.

Many biotechnology derived pharmaceuticals intended for human use have the potential to be immunogenic, especially in animals. During pre-clinical safety assessment it is important to determine if an antibody response has been made against the test substance and to determine if such a response has the potential to be detrimental. One possible outcome is that an immune response to a biological may generate IgE antibodies which can lead to a Type 1 hypersensitivity and the possibility of anaphylactic shock. When an antibody response against a test substance is established it is important to characterize this response and establish possible adverse consequences. In this study we have established a method which can measure IgE mediated release in basophils from cynomolgus monkey whole blood.

White blood cells (WBC) including basophils were isolated from whole blood in ex vivo experiments. Harvested white blood cells were pre-incubated with IL-3, then aliquoted per 100,000 cells. The cal properties of each chemical, molecular weight, boiling point, melting point and Log Kow are not related to the applicability domain of the h-CLAT. On the other hand, many of false-negatives did not dissolve to the medium at the highest applying dose. Therefore, it seems that in the current protocol of h-CLAT, poor solubility of samples to the medium is the most important limitation. We also evaluated the utility of h-CLAT to classify the skin sensitization potential by using various calculated values. From the data of 66 chemicals, which was both positive in h-CLAT and LLNA, several values were calculated as follow: 1) CV75, 2) maximum RFI of each chemical, 3) EC150, and 4) EC200. Correlational analyses between LLNA and the four values were performed. A statistically significant correlation was observed between CV75, EC150, and EC200 values with LLNA EC3. The EC150 value showed the better correlation compared to other values. From EC150 and EC200, Minimum Induction Threshold (MIT) was determined as a minimum value, smallest of either EC150 or EC200. MIT also show the good correlation with EC3. From these data, the h-CLAT might be a one of the useful tool to predict the allergic potency of chemicals after improving the detailed conditions.
of the test substance at 6 different concentrations. Wells containing anti-IgE and compound 40/80 were also tested for each animal as positive controls for IgE and non-IgE mediated histamine release. Histamine release was reported as a percentage of the total cellular histamine content. Although results did not show any clear indication of specific IgE-mediated histamine release towards the test substance, the assay did show reproducible responses when anti-IgE and compound 40/80 were incubated with WBC's (histamine release up to 50% and 30% of total cellular histamine respectively). It is concluded that a valid method to detect IgE and non-IgE-mediated histamine release from buccal epithelium has been developed for use in the cynomolgus monkey.

303 EVALUATION OF PLASMACYTOID DENDRITIC CELL-BASED ASSAY TO DETECTENDEMIC ALLERGENICITY.

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Human dendritic cells have been used to evaluate the allergenicity potential of chemicals and develop alternatives to existing animal models utilized throughout industry to monitor products for contact sensitization. Development of such non-animal alternative assay systems for hazard assessment directly addresses REACH (Registration, Evaluation, and Authorization of Chemicals) legislation. In this study, we investigated whether CD86 expression in plasmacytid dendritic cells (pDC) can be used to identify contact allergens. Human DC were generated from CD34+ progenitor cells and the pDC fraction (CD123+/CD11c+) was harvested using FACS sorting. The pDC were exposed to an expanded list of chemical allergens (n=49) or irritants (n=62). Concentrations of each chemical that resulted in >50% viability as determined by FACS analysis of propidium iodide stained cells were used. Allergens were identified based on stimulation index (SI) calculated by the fold increase in CD86 expression. A preliminary prediction model was developed: materials with SI ≥1.5 in at least 50% of the pDC donors (n=2-5 donors) were labeled as allergens; materials with SI < 1.5 were labeled as non-allergens. Of the 91 materials tested, historical data for 71 materials were available from mouse local lymph node assay (LLNA) and human studies; these data were used to analyze the sensitivity, specificity, and accuracy of the pDC based assay system versus the LLNA method and human response. Evaluation of the pDC method resulted in sensitivity=95%, specificity=81%, accuracy=89%; for the same 71 materials, the LLNA gave sensitivity=84%, specificity=84%, and accuracy=85%. Thus, performance of the pDC was comparable to that of the LLNA. In conclusion, the pDC method appears to be a sensitive and specific predictor of allergenicity. The assay is advantageous because high throughput screening of chemicals is possible, donor-to-donor variation can be monitored, the cells are of human origin, and the assay is considerably more cost effective than the LLNA or other in vivo tests.

304 THE SENSITIZATION POTENTIAL OF FURFURYL ALCOHOL.

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Furfuryl alcohol-based resins are commonly used as binding agents in foundry sand-molds and as a corrosion inhibitor in mortar, grout, and cement. When mixed with foundry sand and exposed to heat or acid catalysts furfuryl alcohol initiates polymerization or “curing” of the sand. As the curing proceeds, it causes the sand to become dimensionally stable and furfuryl alcohol is vaporized. Furfuryl alcohol is considered by the EPA to be a high volume production chemical with over 1 million pounds produced annually in the United States. This coupled with numerous uses provide considerable potential for exposure of workers and the general public to furfuryl alcohol and furfuryl alcohol-based resins. The potential for exposure to furfuryl alcohol exists though pulmonary, oral, and dermal routes of exposure. It has been reported to be highly toxic in laboratory animals with exposure to higher concentrations producing signs of central nervous system depression, such as headache, drowsiness, nausea, and vomiting. Although furfuryl alcohol was nominated and evaluated for carcinogenicity potential by the NTP, studies evaluating immunotoxicity are lacking. Limited human exposure data reports a higher incidence of asthma in foundry mold workers exposed to furan resin, suggesting a potential immunological effect. These studies were executed to evaluate the immunotoxic potential of furfuryl alcohol following exposure including the dermal route. Furfuryl alcohol was tested in a combined irritancy local lymph node assay (LLNA) that also examined irritancy. It was identified to be an irritant at high concentrations and a sensitizer with an EC3 value of 25.6% resulting in classification as a mild sensitizer. Significant increases were observed in the B20+ and IgE+B20+ cell populations in the draining lymph nodes after exposure to furfuryl alcohol concentrations of 25% and 75% respectively. No elevation in total serum IgE levels were observed after exposure to any concentration of furfuryl alcohol. These results suggest that furfuryl alcohol may function as a T-cell mediated sensitizer.

305 IS ORTHO-PHTHALALDEHYDE A SAFE ALTERNATIVE TO GLUTARALDEHYDE?

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Although ortho-phthalaldehyde (OPA) has been recommended as an alternative to glutaraldehyde for the sterilization and disinfection of heat-sensitive medical equipment, its toxicity has not been thoroughly investigated. This pilot study was designed to evaluate the dermal irritation and sensitization potential of OPA. Results of the Epiderm Skin Irritation Test identified OPA as a dermal irritant and further demonstrated that OPA is a more potent skin irritant than glutaraldehyde. Consistent with in vitro results, exposure to 0.75% OPA induced irritancy when evaluated in a combined irritancy local lymph node assay (LLNA) exposed to 0.75% OPA. A concentration-dependent increase in lymphocyte proliferation was observed after OPA exposure with a calculated EC3 value of 0.051%, classifying this chemical as an extreme sensitizer. IgE-inducing potential was evaluated by phenotypic analysis of draining lymph node cells and measurement of total and OPA-specific IgE levels in the mice. The 0.1% and 0.75% exposed groups yielded significant increases in the IgE+ B20+ cell population in the lymph nodes while only the 0.75% exposed group demonstrated significant increases in IL-4 mRNA in the draining lymph nodes and total and OPA-specific serum IgE levels. A significant elevation in OPA-specific IgG1 was also observed after exposure to 0.75% OPA. These results demonstrated the dermal irritation and sensitization potential of OPA in an animal model raising concern about the skin irritation and sensitization potential of OPA among healthcare workers who are potentially exposed to the chemical.

306 COMPARISON OF CONTACT ALLERGEN-INDUCED GENE EXPRESSION CHANGES IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL-DERIVED DENDRITIC CELLS AND THE DENDRITIC CELL SURROGATE CELL LINE MUTZ-3.

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Since the use of dendritic cell surrogate cell lines in the development of in vitro skin sensitization test methods holds several advantages over the use of peripheral blood mononuclear cell-derived dendritic cells (PBMC-DC), expression analysis of 29 potentially predictive genes derived using PBMC-DC were evaluated in the DC surrogate cell line MUTZ-3. A pilot set of 5 allergens (DNBS, 2-MBT, CAL, EUG, ISOEUG) and 3 non-allergens (SA, LA, SLS) were tested in two independent experiments in the MUTZ-3 cells. If a gene response was positive to allergen treatment or negative to a non-allergen treatment in at least one of the two experiments it was considered as positive or negative, respectively. The response of the cell line to treatment with the non-allergens was predominantly negative. In the MUTZ-3 cells the only ‘false’ positive gene expression changes (CCL2, ILR, and MRC1) occurred following treatment with salicylic acid. Overall the response of the cell lines to non-allergen treatment was more predictive than the response of PBMC-DC which had 12 genes with false positive responses to salicylic acid treatment and two false positive genes each with lactic acid and sodium lauryl sulfate. For the MUTZ-3 cells 6 genes failed to respond to any of the 5 test allergens. In comparison, 17 of the 29 genes were positive with all 5 allergens in the PBMC-DC. This is not surprising since the gene list was developed based on the response of PBMC-DC. The best performing gene with the MUTZ-3 cells was CCL4 which was positive for all five allergens and negative for the three non-allergens. A number of other genes were positive in the MUTZ-3 cells with four of the five allergens and negative with all non-allergens: ARHGIDB, CD1E, CTSH, EPB41L2, S100A4 and SLAM. The MUTZ-3 cell line shows some promise as a DC-surrogate for use in a gene expression-based method. However additional testing with an expanded test set of chemicals will be needed.

307 RECONSTRUCTED HUMAN EPIDERMIS INTEGRATING LANGERHANS CELLS (RHE-LC) RESPONSE TO CONTACT SENSITIZERS.

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The most common manifestation of immunotoxicity in humans is allergic disease resulting from industrial or environmental exposure to sensitizers. A range of different in vitro models have been used, in order to understand the mechanisms through which chemical allergens induce allergic contact dermatitis in humans, and to develop in vitro assays to assess the potential of a chemical to induce skin sensitization
without the need of animal testing. However, none of them efficiently simulate the natural tridimensional microenvironment in which the main cellular actors of skin sensitization, dendritic cells (DC) and keratinocytes (KC), co-exist like in the skin. We present here a full characterization of such a model, consisting in a reconstructed human epidermis integrating CD34+ derived Langerhans cells, and we assess its response after exposure to TNF-α or to three contact sensitizers (DNCB, pPD and Oxazoline). The results show that TNF-α treatment induces a significant and reproducible increase of the expression of three genes encoding specific markers of mature DC: CCR-7, CD80 and CD83. The expression of these three genes is also induced in response to sensitizers. However the response profile varies depending on the sensitizer and its application mode (topical or systemic). Overall we conclude that the RHE-LC model is functional and allows to study the response to sensitizers in a tridimensional co-culture model. This will result in a better understanding of the cross-talk between KC and DC during chemical exposure and of the role of each cell type in the response to a sensitizer.

**308 PEPTIDE REACTIVITY PROFILES OF REFERENCE CONTACT AND RESPIRATORY LOW MOLECULAR WEIGHT CHEMICAL ALLERGENS.**

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Low molecular weight chemicals are capable of causing allergic diseases of the skin and respiratory tract. Despite opportunities for exposure to both organs, individual materials are typically only associated with one form of disease or the other. The reasons for this divergence are not known; however, it is clear that chemical modification of proteins is an important common step for the initiation of allergic responses. The amino acid reactivity of a reference skin sensitizer (2,4-dinitrochlorobenzene [DNCB]) and a reference respiratory allergen (toluene-2,4-diisocyanate [TDI]) was investigated. The assays were conducted by preparing a reaction mixture of synthetic peptides containing cysteine (Cys), lysine (Lys), tyrosine (Tyr) or histidine (His) with an excess of test chemical. Dose response evaluations were conducted at peptide to test material ratios of 1:10, 1:25 and 1:50 and samples incubated for 24 h. Kinetic evaluations were conducted at a ratio of 1:50 with analysis from 3 min to 24 h. The reactions were analyzed by HPLC/UV (and/or MS). The data were expressed as the percent depletion of peptide from the reaction compared with the controls. DNCB and TDI were observed to exhibit differing potentials to react with specific amino acids. The rank order of maximum reactivity at 24 h (based on percent depletion) with DNCB was Cys (100%) > Lys (21%) > Tyr (46%) > His (0.9%). For TDI, the rank order was Cys (88%) > Lys (26%) > His (6%) > Tyr (0.2%). Examining the kinetic profiles revealed further differences, where similar reactivity was observed at the 24 h time point. For example, TDI reacted rapidly with lysine (maximal reactivity within 3 minutes), whereas DNCB reacted linearly over the 24 h period. These observed differences in peptide reactivity profiles may be of importance for the ability of these chemicals to induce divergent allergic responses.

**309 TRICHLOROETHENE INDUCES APOPTOSIS IN HEPG2 CELLS AND IMPAIRS CLEARANCE OF APOPTOTIC CELLS BY RAW CELLS.**

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Trichloroethene (TCE) is an environmental pollutant that has been implicated in autoimmune diseases. Earlier, we reported that female MRL+/+ mice given TCE chronically in the drinking water (0.5 mg/ml) develop autoimmune hepatitis. Increased lymphocytic infiltration with areas of mild necrosis was seen after 48 weeks by H&E staining of liver sections. Significant increase in apoptosis was noticed after 24 weeks as compared to 36 and 48 weeks by TUNEL staining. Kaplan cells showed a compromised clearance of apoptotic bodies after 36 and 48 weeks. Therefore, we hypothesized that decreased clearance of apoptotic bodies leads to secondary necrosis and inflammation. To test this hypothesis, we incubated human hepatoma cells (HepG2) with TCE at different concentrations (1 to 32 mM) for various lengths of time (1 to 72 hours). A decrease in cell viability was found by MTT assay at 12 mM and higher concentrations after 24h. A significant increase in apoptosis was also observed following exposure to 12 mM or higher concentrations of TCE by both TUNEL assay and flow cytometry (Annexin and propidium iodide staining). A decrease in phagocytosis of TCE-treated HepG2 cells by RAW (murine macrophage) cells was also observed. These studies indicate that TCE induced apoptosis and their compromised phagocytosis, can be a potential mechanism for the induction of autoimmune hepatitis.

**310 MICE EXPOSED TO A BINARY MIXTURE OF IMMUNOTOXICANTS DEVELOPED UNIQUE AUTOIMMUNE EFFECTS NOT INDUCED BY SINGLE EXPOSURE.**

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People are rarely exposed to individual chemicals at levels known to induce overt toxicity. However, little is known about the response to low-level chemical mixtures, a scenario that more commonly mimics actual human exposure. Chronic low-level exposure to the common water contaminant trichloroethylene (TCE) has been linked to the development of autoimmune disease in humans. Similarly, MRL+/+ mice exposed to TCE for 32 weeks in their drinking water develop autoimmune hepatitis. The current study was designed to examine whether co-exposure to another toxicant, mercuric chloride (HgCl2) altered TCE-induced autoimmune hepatitis. Female MRL+/+ mice were treated for only 8 weeks with TCE (0.1 mg/ml in drinking water) +/- HgCl2 (40 μg sc twice a week). Unlike the mice exposed to either TCE or HgCl2 alone, mice exposed to both toxicants developed early stages of autoimmune hepatitis after only 8 weeks. Disease development in the co-exposed mice was accompanied by a unique pattern of anti-liver and anti-brain antibodies not found in the blood of mice exposed to either toxicant alone. These antibodies recognized high molecular weight (between 75-100 kDa) proteins. Unlike single exposure co-exposure to TCE and HgCl2 also stimulated expression of pro-inflammatory cytokines (e.g. IL-1β). Thus, exposure to a binary mixture of immunotoxicants induced a unique immune response and accelerated autoimmune disease development.

**311 N-ACETYLCYSTEINE SUPPLEMENTATION PROTECTS AGAINST TRICHLOROETHENE-INDUCED AUTOIMMUNITY: ROLE OF OXIDATIVE STRESS.**

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Mechanisms by which trichloroethene (TCE) induces an autoimmune response are not well understood. Previous studies from our laboratory suggest that oxidative stress (increased lipid peroxidation and protein oxidation) may contribute to TCE-induced autoimmunity. The current study was undertaken to further assess the role of oxidative stress in TCE-induced autoimmunity by supplementing N-acetylcysteine (NAC), an antioxidant, through drinking water. Groups of female MRL+/+ mice were treated with TCE, NAC or TCE plus NAC for 6 weeks (TCE, 100 mg/kg, i.p., every 4th day; NAC, 250 mg/kg/day through drinking water). TCE treatment led to significant induction of anti-malondialdehyde (MDA) and anti-hydroxynonenal (HNE)-protein adduct antibodies along with increased carbonylation of serum proteins, suggesting an increase in oxidative stress. The TCE-induced oxidative stress was also associated with significant increases in serum levels of anti-nuclear-, anti-Sm- and anti-dsDNA-antibodies. Interestingly, NAC supplementation not only attenuated the TCE-induced oxidative stress but also the markers of autoimmune response, as evidenced by their reduced levels in the sera of TCE plus NAC treated mice. These findings further support a role of oxidative stress in TCE-induced autoimmune response. Attenuation of autoimmune response by NAC could be important in developing preventive strategies for ADs. Supported by NIH ES016302.

**312 DIRECT ACTIVATION OF B1A B CELLS VIA ASBESTOS EXPOSURE.**

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Exposure of the immune system, specifically the B1a B lymphocyte population, to silicates such as crystalline silica or asbestos has been implicated in the production of auto-antibodies and ultimate development of systemic autoimmunity in both mice and humans; nevertheless, the etiology of the autoimmune response remains indistinct. B1a B cells primarily reside within the peritoneal and pleural cavities.
and they have the ability to self-renew without egression to lymphoid germinal centers. This maintains the hypothesis that these cells are not examined by the mechanisms which suppress the release of auto-antibody producing B cells; thus, the additional activation upon exposure to asbestos may cause increased B1a B cell trafficking and production of T-independent IgM, some of which may be auto-reactive. Nevertheless, the knowledge pertaining to the mechanism of how asbestos exposure accomplishes such a reaction has yet to be deciphered. To test the hypothesis that asbestos affects the B cell line CH12.LX in a direct manner, approximately 1 x 10⁶⁴ cells were exposed to asbestos in 10 μg/cm², 25 μg/cm², and 50 μg/cm² quantities. Following exposure the cells were processed using the CyQuant Proliferation Kit. The results acquired from this assay demonstrated that asbestos in a 50 μg/cm² concentration caused enhanced cell proliferation. Further testing of the hypothesis was performed using flow cytometry to detect the production of interleukin 10, but they also traffic to secondary sites, such as the spleen, as indicated by the decrease in integrin protein. We conclude that B cells from cell line CH12.LX and from the peritoneal cavity are directly activated to proliferate and traffic to secondary sites upon exposure to asbestos.

313 SERUM CYTOKINE/CHEMOKINE PROFILE IN PENICILLINAINDUCED AUTOIMMUNITY.

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Rationale: At present, idiosyncratic drug reactions (IDRs) are unpredictable largely due to a lack of mechanistic understanding but their clinical characteristics suggest that they are immune-mediated. Penicillin-induced autoimmunity in Brown Norway rats has been utilized as an animal model for mechanistic studies of one type of IDR because it mimics the autoimmune syndromes it causes in humans. The aim of this study was to gain further mechanistic insights into this IDR from its serum cytokine/chemokine profile. Experimental Procedures: BN rats were given penicillin (1.5 mg/ml in drinking water). Blood samples were collected once a week and 24 cytokines/chemokines were determined by Lumexin. In addition, serum concentrations of IL-6 and IL-22 were determined on days 1, 3, 5 and 7 by ELISA. Results: 15/20 treated rats developed autoimmunity. In sick animals, IL-6 and TGF-beta1, known to be driving forces of Th17 differentiation, were consistently increased shortly before the onset of autoimmunity and a few days after the treatment, respectively. IL-17, one of the most characteristic cytokines of Th17 cells, was increased in sick animals at both early and late time points. The early peak is likely from innate immune cells. Meanwhile, IL-22, another signature cytokine of Th17 cells was elevated before the onset of autoimmunity. This suggests the involvement of Th17 in pathogenesis of penicillin-induced autoimmunity. The primary increase in IL-6 and IL-22 on day 1 and 3, respectively, provided additional evidence in support of penicillin-induced Th17 cell development. Conclusions: Our data provide important mechanistic clues that may help to predict which drug candidates will cause a relatively high incidence of such autoimmune IDRs. This work was supported by a grant from the Canadian Institutes of Health Research.

314 INVESTIGATION OF THE DANGER HYPOTHESIS IN AROMATIC AMINE DRUGS.

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Sulfamethoxazole (SMX) is an aromatic amine antioxidant associated with a high incidence of immune-mediated idiosyncratic drug reactions (IDRs). In general, aromatic amines are toxic, presumably because they can be oxidized to reactive metabolites that can bind protein and redox cycle to produce oxidative stress. The danger hypothesis postulates that cell stress responses are triggered and if true, danger signals could be biomarkers of IDR risk. A previous study in mice to test the danger hypothesis found that SMX, the only aromatic amine tested, was also the only drug not associated with the upregulation of genes associated with cell stress and most changes involved down regulation. However, metabolism of SMX in mice is limited. To ensure that these observations were not specific to mice and to determine if down regulation of gene expression is a biomarker of IDR risk common to aromatic amines, the experiment was repeated in rats and two other aromatic amine drugs were included: dopamine (DDS) and aminoglutethimide (AMG). DDS is similar in structure to SMX but AMG is significantly different with a higher electron density. Changes in hepatic gene expression were determined with Affymetrix microarrays. As in mice, SMX induced minimal changes in gene expression and none suggested cell stress. DDS and AMG induced greater gene changes than SMX including the upregulation of enzymes involved with oxidative stress and drug metabolism such as aldo-keto reductase, glutathione S-transferase, and aldehyde dehydrogenase, which may represent danger signals. Serum- and glucocorticoid-regulated kinase gene expression was significantly down regulated in all three drugs. Preliminary results for protein expression of chemokine (C-X-C) 1 and thymic invades 1, using ELISA, were consistent to gene expression for SMX and may play a role in early stress signalling. Thus, a common expression profile for aromatic amine drugs was unclear and these results suggest that finding biomarkers to predict IDR risk is complex, even for structurally similar drugs. This research was funded by grants from the Canadian Institutes for Health Research.

315 COVALENT BINDING OF NEVIRAPINE IN VIVO AND IN VITRO.

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Rationale: Nevirapine (NVP) treatment is associated with significant idiosyncratic and immune-mediated skin rash and hepatotoxicity. The central hypothesis is that development of skin rash and liver toxicity are mediated by covalent binding of a NVP metabolite to protein. This study was designed to examine the covalent binding of NVP and other metabolites to skin and liver tissues. Methods: Anti-NVP antibody was produced in New Zealand White Rabbits and used in immunoblotsing and immunochemistry studies to detect covalent binding of NVP to liver, skin, and ear proteins. Samples were obtained from Brown Norway rats treated with NVP (150 mg/day; 8 days) or various analogs in vivo, and from in vitro incubations using rat microsomes or homogenized skin and ear tissue. Results: Hepatic covalent binding in NVP-treated animals was greatest in the centralobular region. Co-treatment with aminobenzotriazole decreased binding and altered its pattern. Several proteins were modified and ranged from 40 to 83 kD. Binding was also observed when NVP was incubated with expressed CYP3A and 2C11. Covalent binding to hepatic microsomal NVP was significantly lower for the deuterated NVP analogue and 12-OH-NVP than for NVP. Homogenized rat skin incubated with 12-NVP-sulfate produced a faint band at 25 kD, but no binding was observed in an incubation with NVP. Conclusions: The reactive metabolite that led to covalent binding in the liver is likely to be a quinone methide formed by oxidation of the methyl group; however, it does not appear that this occurs in skin. It is more likely that covalent binding in the skin involves 12-NVP-sulfate. Oxidation of the NVP methyl group is required for the formation of both reactive metabolites which is consistent with the decreased risk of rash associated with the analog in which the methyl hydride were replaced by deuterium. This research was funded by grants from the Canadian Institutes of Health Research.

316 DANGER SIGNALS IN NEVIRAPINE-INDUCED SKIN RASH.

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Background: The HIV drug, nevirapine (NVP) is associated with a high incidence of skin rash and liver toxicity. The mechanism of NVP-induced skin rash has been studied in our animal model in which NVP-treated Brown Norway (BN) rats develop an immune-mediated skin rash with characteristics very similar to the skin rash observed in humans. Oxidation of NVP to the 12-hydroxy-metabolite (12-OH-NVP) is required, presumably because it is further metabolized to a more reactive sulfate. The goal of this study was to determine the mechanism by which this metabolite induces an immune response; specifically, to determine if it involves the production of a danger signal. Since 12-OH-NVP induces a skin rash at a lower dose than NVP and the analogue of NVP in which the methyl hydrogens are replaced by deuterium (DNVP, this inhibits 12-OH-NVP formation) did not induce skin rash, we attempted to use 12-OH-NVP and DNVP as positive and negative controls, respectively, to detect potential molecules that are responsible for the induction of skin rash.

Methods and Results: Female BN rats were treated with NVP 12-OH-NVP and DNVP at 6 hour after changes in gene expression in skin from the ears were determined with Affymetrix gene chips. Nm4k, an early response gene, was highly upregulated in 12-OH-NVP treatment but not by DNVP treatment. Other interesting changes in gene expression involved $100 a7a and CEBPδ. Although HMGB-1 gene expression did not change, its activity is controlled by acetylation, and we found an increase in HMGB-1 with a lower isoelectric point in NVP-treated animals, presumably reflecting acetylation.
Conclusions: These results are consistent with the danger hypothesis. Future experiments will involve modification of these genes to determine their role in the induction of skin rash by NVE. This research was supported by grants from the Canadian Institutes of Health Research.

317 VALIDATION OF AN ORAL EXPOSURE MOUSE MODEL FOR THE PREDICTION OF DRUG HYPERSENSITIVITY REACTIONS USING A REPORTER ANTIGEN.

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Adverse drug reactions (ADRs) are the main cause of black box warnings or even drug withdrawals. Due to their idiosyncratic (and therefore rare) nature, ADRs are often not noticed until a drug has been marketed and used in the general population for some time. So far there are no validated in vitro or in vivo models to predict possible ADRs and there is a demand for pre-clinical screening tools. Previous studies have shown the possible predictive value of an oral mouse model using the Reporter Antigen (RA), Triinitrophenyl-Ovalbumin (TNP-OVA). Using this model, the sensitizing capacity of e.g. Diclofenac (DF) and D-Penicillamine (D-Pen) have been demonstrated. In the current study we extended this data by testing the analgesic drug acetaminophen (APAP) and its non hepatotoxic regioisomer (AMAP), the antibiotic Oloxacin (OFLX), and the anti-diabetic drug Metformin (MET) in the oral model using TNP-OVA as read out. We evaluated whether these compounds were able to cause sensitization via oral exposure. Therefore, C3H/HeOuJ mice were dose by oral gavage (30/100/300mg/kg bodyweight for APAP and AMAP, 100/300/1000mg/kg for OFLX and 50/100/500mg/kg for MET) for 7 consecutive days. At the first day of exposure the mice received an intraperitoneal injection of TNP-OVA. After 15 days they were ear-challenged with TNP-OVA and Delayed Type Hypersensitivity (DHT) was assessed 24 hours later. One week after challenge, the draining lymph node was removed and TNP-specific antibody secreting cells (ASC) were determined. Serum collected on day 21 was analyzed for TNP-specific antibodies. Both APAP and OFLX showed a significant dose dependent increase in DTH responses to ear-injection with TNP-OVA, whereas both AMAP and MET did not. ASC and serum antibodies against the RA were only detected in some individual drug treated mice. In conclusion, the present oral exposure model has the ability to identify drugs known to cause adverse immune reactions, with the increase of a DTH response as most important parameter.

318 COMBINATION TOXICITY STUDIES FOR PHARMACEUTICAL AGENCTS: DESIGN CONSIDERATIONS AND IMPACT ON CLINICAL DEVELOPMENT.

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Drugs or biotherapeutics are often combined in the clinic to maximize efficacy. The impact of such combination therapies are well known in the field of oncology and viral therapy, specifically HIV. The benefits of combination therapies have influenced pharmaceutical industries to explore development of new molecular entities (NME) with either NMEs or marketed products, or the combination of marketed products. In 2006, the Committee for Medicinal Products for Human Use (CHMP) and the U.S. FDA issued guidelines for nonclinical safety evaluation for combination products. The need for combination toxicity studies are dependent on the existing clinical and nonclinical data for each individual compound that is used to support the proposed dose and duration in patients. Nonclinical combination toxicity studies are generally conducted to evaluate whether combination of two or more agents cause a potentiation, synergistic, or additive effects on target organ toxicities that were identified for individual compounds. Design of such studies is critical in hazard identification as it impacts clinical monitoring. Usually the most sensitive species is used. Dose selection for individual compounds should consider levels that have some minimal effect so that exacerbation or additive effects can be clearly evaluated. Usually establishment of a NOAEL is not necessary, unless it is being developed as a co-formulation and there is lack of clinical/nonclinical data on individual compounds. Duration of studies depends on the type of toxicity profile that is seen with individual compounds and usually does not exceed more than 90 days of dosing. Integration of data from ADME, PK, and clinical studies for individual compounds is important for designing successful combination toxicity studies. The roundtable will discuss general considerations for when and how to conduct combination toxicity studies with special focus on design considerations and challenges. Case examples and shared learning from combination toxicity studies and the impact on clinical monitoring will be discussed.

319 MELAMINE CONTAMINATION OF INFANT FORMULAS: LESSONS LEARNED.

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In September 2008, officials in China acknowledged that illegal use of a fraudulent protein substitute, melamine, for months had contaminated powdered infant formulas sold throughout the country. The tainted formulas also entered the market in several countries, in South East Asia and Africa. In the Peoples Republic of China alone at least 51,900 children are believed to have been affected by tainted food products, of whom 6 died. Some milk products contained > 2000 ppm melamine. In 2007 pet food contaminated with melamine, cyanuric acid, ammeline, and ammelline affected thousands of cats and dogs in North America. In the pet food outbreak, the pathogenesis involved co-precipitation of melamine and cyanuric acid in renal distal tubules and collecting ducts, causing acute renal failure. In the infant formula outbreak, the cardinal toxic effect of melamine in these children was the presence of kidney calculi, leading to acute kidney injury. Unlike in pets where melamine-cyanurate interaction was a significant phenomenon in pathogenesis of acute renal failure, in infants the crystals consisted of melamine alone. The mechanism of melamine-induced nephrotoxicity in infants remains unknown. This emerging disease is likely to occur again because of the widespread use and availability of melamine and its analogues in the environment. The objectives of this session are to offer the current scientific status of this tragedy arising from the unscrupulous use of melamine in infant formula and how to use this knowledge to develop better public health and safety policies. Presentations will cover comparison between renal failure in pet food and infant formula outbreaks, dose-response, and risk assessment considerations of melamine in infants, the chemistry and analysis of melamine and analogues in food for risk assessment purposes, guidance on levels of health concern in foods, and regulatory aspects.

320 TRANSLATING TOXICOLOGY TO PUBLIC HEALTH PROTECTION: LESSONS LEARNED FROM SUPERFUND.

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The NEIHS strives to improve human health through the translation of scientific discoveries from bench to policy and bench to public health. The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) defined and refined how toxicology can translate to public health benefits. This includes using the best available science to make health protective decisions at Superfund sites and conversely adapting what is learned at sites to inform new research directions. This two-way communication channel is exemplified by the NEIHS Superfund Research Program (SRP). The SRP, mandated by Congress to complement the applied nature of the national Superfund program, supports teams of scientists from the biomedical, engineering, environmental, and ecological disciplines to provide fundamental knowledge that could be used by decision-makers. To accelerate the timeframe whereby science is used by decision-makers, each SRP must include translational activities which include technology transfer, community outreach, and partnerships with governmental agencies. Superfund responses need to act on the best available science, and not be halted by knowledge gaps in toxicology. This session will examine what lessons have been learned from the SRP, how toxicological research can be translated to remediation decisions; how biomarkers can inform risk assessment; how biomonitoring can reduce exposure at contaminated sites; and how SRP innovation can benefit the multi-agency work at Superfund sites.

321 PROTECTIVE EFFECTS OF PHENOCYONATE HYDROCHLORIDE AGAINST NERVE AGENT-INDUCED SEIZURE ACTIVITY.


This study utilized the guinea pig nerve agent-seizure model to measure the anticonvulsant efficacy of phenocyonate HCl (PCH), a compound that possesses a combination of anticholinergic and anti-NMDA activities, and compared its effectiveness with that of scopalamine (SCP) at early (at time of seizure onset) and late (at 40 min after seizure onset) phases of seizure progression. Animals were implanted with cortical electrodes for electroencephalographic (EEG) recordings one week earlier. On the day of the experiment animals were pretreated intramuscularly (im) with pyridostigmine bromide (0.026 mg/kg) 30 min prior to exposure to a 2.0
x LD50 subcutaneous (sc) dose of a nerve agent (GB, GD, GF, or VX). One minute after a nerve agent, animals were treated im with atropine sulfate (0.1 mg/kg) and 2-PAM (25 mg/kg) and were observed for the onset of EGG seizures. At the onset of seizures (early phase) or at 40 min after seizure onset (late phase), animals were treated with a dose (range 1.0 – 10.0 mg/kg, im) of PCH or SCP. The anticonvulsant ED50 doses of these two treatments individually against each nerve agent were determined. When administered at seizure onset PCH provided equivalent anti-convulsant effects in comparison to SCP against GB and VX intoxication, while SCP was significantly more potent than PCH against GF intoxication. At 40 min after seizure onset following GD intoxication PCH or SCP administration was equally effective as an anticonvulsant treatment. PCH provides excellent anti-convulsant efficacy against nerve agent-induced seizure activity when administered at early or late phases of seizure progression, and these effects are equivalent to or slightly less potent than those of SCP therapy.

322 CYTOTOXICITY OF CYCLOTRIMETHYLENETRINITRAMINE (RDX) IN PRIMARY HUMAN CELL CULTURES.

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The explosive cyclotrimethylenetetranitramine (RDX) has been found to produce convulsions and tremors in animals and in humans, suggesting that the compound may have neurotoxic potential. To further understand the toxicity observed in vivo, a comprehensive in vitro evaluation of RDX cytotoxicity was performed with human primary cells (hepatocytes, renal proximal tubular epithelial cells, aortic endothelial cells, dopaminergic neurons, astrocytes and microglia cells. The cells were evaluated as conventional single cell type cultures and co-cultures as Integrated Multiple Organ Co-culture (IdMOC). Cellular ATP content, MTT metabolism, and caspase 3/7 activation were used as endpoints. As single cell type cultures, the primary cells were cultured in collagen-coated 96-well plates. The IdMOC contained six types of cells (all except the aortic endothelial cells) as physically-discrete cultures interconnected by an overlying medium. Both single cell type cultures and IdMOC were treated with RDX at concentrations of 3.5 to 222 μg/mL (single cell type cultures) and 8.9 to 222 μg/mL (IdMOC) for 24 hr. With the single cell cultures there was a trend of limited dose-dependent decrease of cell viability, with the cellular ATP content as a more sensitive endpoint than MTT metabolism. The maximum decrease in viability based on ATP was <30% (>70% relative viability). Similar results were observed with the IdMOC co-cultures. No caspase 3/7 activation was observed with the single cell type cultures or IdMOC, suggesting no apoptotic effects. These results show that RDX has no or at most extremely weak cytotoxic potential towards human primary cells, thereby suggesting that the seizures observed in vivo may not be a result of cellular cytotoxic events.

323 COMPARISON OF EFFECT UPON AND RECOVERY OF CHOLINESTERASE (CHE) ACTIVITY IN TISSUE AND BLOOD AFTER REPEATED INHALATION OR SUBCUTANEOUS SARIN (GB) EXPOSURES.

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Study objective was to collect Che activity data following subcutaneous (SC) and whole body inhalation (IH) exposures to 3 GB sub-lethal doses day apart in guinea pigs to improve PBPK/PD models. Tissue & blood Che activity was collected & evaluated at sequential intervals after exposures at 2 different concentrations (0.1/0.4LD50). With repeated 0.4LD50 doses RBC AchE remained depressed but showed recovery signs 24 hr after each exposure; but repeated 0.1LD50 exposures had minimal effect upon AchE activity. With 0.1LD50 SC/IH dose, BChE activity was marginally depressed after multiple exposures at 24 hr each after first 0.4LD50 dose BChE activity was ~80% inhibited, appeared to recover & was inhibited similar amount following subsequent doses. In tissues at 48/72 hr after both IH doses brain & eye AchE activity was most affected (34-65% ctrl). Diaphragm AchE activity was moderately affected (72-74% ctrl). Higher IH exposure reduced lung AchE activity (62-65% ctrl). Only at 72 hr after higher IH exposures were kidney & liver AchE activities moderately inhibited (70-75% ctrl). At both GB exposures heart AchE activity was relatively unaffected. At 48/72 hr after 0.1LD50 SC exposure, all tissues except diaphragm (44% ctrl at 48 hr) approached control values; however, 96 hr after exposures, brain, eye, lung, heart & diaphragm AchE activities were 34-51% of controls. At the 0.4LD50 SC exposure, brain, eye, lung, heart & kidney AchE activities were inhibited at 48 & 72 hr post-exposure; while brain, eye, lung, heart, diaphragm & liver AchE activities were still inhibited (26-60% ctrl) 96 hr post-exposure. In certain tissues (brain, eye and lung for both exposure routes and heart for SC route only) AchE recovery after GB exposure occurs at slower pace compared to recovery in RBC. Also following SC exposure in diaphragm & liver a lag time for AchE activity inhibition occurs. Data generated is relevant to force health protection concerns that include individual protection, de-contamination & environmental surveillance.

324 REACTIVATION OF PHOSPHORYLATED ACETYLCHOLINESTERASE IN THE CENTRAL NERVOUS SYSTEM USING NOVEL PYRIDINIUMOXIMES.

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Inhibition of acetylcholinesterase (AChE) due to organophosphate (OP) poisoning traditionally is treated using atropine sulfate and an oxime-like 2-PAM. Currently available oximes have limited abilities to cross the blood-brain barrier (BBB), resulting in little, if any, reactivation of brain AChE. This study involves testing pyridinium oximes, synthesized to incorporate moieties which increase lipophilicity and BBB penetration. The new oximes, along with 2-PAM, were tested in vitro and in vivo to determine the optimal reactivator AChE inhibition by experimental OPs (synthesized novel sarin surrogates). The in vitro assay OP phthalimidyl isopropyl methylphosphonate (PIMP: IC50~36nM), leaves AChE phosphorylated with the same moeity as sarin, but rapidly degrades in aqueous solution, preventing re-inhibition of reactivated AChE. Rat brain homogenate was treated with PIMP (80% inhibition), followed by 0.1nM oxime, and AChE activity measured by discontinuous spectrophotometric assay. Reactivation varied among oximes (24–78%), with 2-PAM yielding 89%. Lipophilicity was determined by n-octanol/water partition coefficients and varied among oximes (ranging up to 0.680) with some over 100-fold higher than 2-PAM (0.006). Oximes with enhanced lipophilicity and reactivation potential above 40% were selected for in vivo studies. Adult rats were treated with a stable analog of the experimental OP nitrophenylo isopropyl methylphosphonate (NIMP; 70-80% brain AChE inhibition), followed by im administration of 2-PAM or selected oxime. Additional changes in brain chemistry due to NIMP are also being tested. As expected, 2-PAM did not reactivate brain AChE; however, a few novel oximes yielded 10-30% reactivation. These results indicate therapeutic potential of these oximes toward reactivation of sarin phosphorylated brain AChE. Supported by Defense Threat Reduction Agency: 1.E0056_08_AHB_PP_C.

325 APPLICATION OF A PBPK/PD MATHEMATICAL MODEL FOR THE OXIME HI-6 TO RELATED COUNTERMEASURES: HLo7, MM-4, TMB-4 AND OXIDOXIME.

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Many oximes have been synthesized and evaluated for use as countermeasures against chemical warfare nerve agents. Novel oxime development is complicated by the tremendous variations in species efficacy and effectiveness against different agents. To facilitate the understanding of these variations and provide a rational mechanistically-based approach for extrapolating efficacy estimates to humans, a physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model was developed based on a validated HI-6 model. The code was expanded to determine if other relatively well known oxime kinetics and dynamics could be described. The oximes tested include another Hagedorn oxime, HLo7, as well as the older oximes, methoxime (MMB-4), trimedoxime (TMB-4) and oxidoxime. Literature data sets were simulated for multiple species, including rats, pigs, African green monkeys and humans. Partition coefficients for the oximes were predicted using structure-activity relationship algorithms and refined using limited TMB-4 tissue concentrations. Preliminary results show good prediction of oxime kinetics across species and compounds. Ultimately, the model will include pharmacodynamic effects, includ-
ing oxime reactivation and organophosphate dissociation. This PBPK/PD mathematical model will allow in silico testing of countermeasure dosing regimens and facilitate extrapolation of novel oxime effects in experimental animals to humans. This project received support from the Defense Threat Reduction Agency - Joint Science and Technology Office, Basic and Supporting Sciences Division.

326 EVALUATION OF OPTIMIZED MONOCLONAL ANTIBODIES AGAINST STAPHYLOCOCCAL ENTEROTOXIN B BY IN VITRO PROLIFERATION AND MURINE SHOCK MODELS.

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Staphylococcal enterotoxin B (SEB), an exotoxin produced by Staphylococcus aureus, is classified as a bacterial superantigen (SAg) and is also considered a biologically threat agent. Once internalized, SAg bypass normal antigen presenting cell processing and binds directly to variable β-chain groove of circulating T cells via MHC-II receptors, resulting in a profound acute inflammatory response that can produce significant system-wide pathologies. Epitope-optimized monoclonal antibodies (mAbs) hold the greatest therapeutic potential for in vivo steric hindrance of SEB-induced inflammation in the event of power release. We screened a library of mAbs to inhibit SEB (1 μg/mL) induced proliferation using fresh-derived PMBCs from healthy rhesus macaques (M. mulatta) and selected mAbs that produced a significant reduction (>50%) of SEB-induced proliferation. Thereafter, to assess in vivo efficacy of the selected mAbs, BALB/c mice were exposed either by oral gavage, small-particle aerosol, or intraperitoneally to a multiplicity of predetermined route-specific lethal doses of SEB followed by a 75 μg injection of lipopolysaccharide as a potentiating agent. Results indicated that the oral and inhaled murine model, when potentiated by LPS, was remarkably similar in clinical immunological development and pathologically to primate models of SEB intoxication. Similarly, the SEB aerosol model showed lung and cytokine pathology as prior model development both in the primate model as well as susceptible (tG HLA-DQ8+) mice. Overall, these studies indicate that in vitro screening preceding in vivo evaluation of mAb-based therapies for SEB is appropriate for early identification of products that may be of clinical use for SAg-induced shock.

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327 MECHANISTICALLY-BASED IN SILICO SIMULATION OF PATHOGEN-HOST IMMUNE RESPONSE DYNAMICS.

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There is a need to quantify the relationship between biotreatable exposure and human physiological response, to predict the effects of exposure. Currently we rely on animal experiments to characterize the exposure-response relationship. However, animals may fail to mimic aspects of the human infectious process, and humans may be more or less susceptible to infection. Biologically-based models developed for animals and humans that incorporate aspects that differ between the species (e.g., airway deposition, toxin kinetics, immune response) can be used to correct for these differences and extrapolate animal data to humans. Although various infectious organisms and their mechanisms of pathogenesis are distinct, they all are capable of subverting the host immune response through interference with signaling/activation of adaptive immunity. A biologically-based modeling approach that incorporates these mechanisms and allows for the changing of cellular and chemical targets is global and applicable to multiple agents. The current model consists of a simplified immune system module, with several cell type populations (e.g., phagocytes, T cells, B cells) and a simple set of signaling and communication pathways to represent the actions of various cytokines and cellular crosstalk. Competing interactions between pathogens and immune system components are incorporated in the model, allowing simulations and predictions of the outcome of exposure and/or infection. Simulations with the preliminary model were conducted with various starting conditions (e.g., levels of microorganism/phagocytes) to examine the behavior of the system. Results suggest that coupling the model with physiologically-based inhalation and compartmental systemic models will allow simulation of real world exposure scenarios. This integrated model will provide a mechanistically-based, rational approach for predicting the impact of human exposure to pathogens.

328 TUNGSTATE EXPOSURE REDUCES THE QUANTITY OF CYTOTOXIC AND HELPER T-CELLS IN C57BL6 MICE AFTER IMMUNE CHALLENGE.

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Tungstate (WO₄⁻) has been identified as a water contaminant and can be absorbed by ingestion. In this study, C57BL6 mice were exposed to tungstate (as Na₂WO₄.2H₂O) or their drinking water for either 28 or 90-days. Drinking water included weight adjusted Na₂WO₄.2H₂O concentrations of 0, 62.5, 125, and 200 mg/kg/day. Twenty-four hours prior to sacrifice, mice were given (i.p.) saline, lipopolysaccharide (LPS) (5 mg/kg), or Staphylococcus aureus enterotoxin B (SEB) (20 μg) to stimulate immune responses. After sacrifice, splenocytes, blood, bone marrow and thymus were immunophenotyped with lymphocyte and/or myeloid antibody panels. Flow cytometric analysis revealed that splenic cytotoxic T-cells (CD3⁺CD8⁺) and helper T-cells (CD3⁺CD4⁺) of the 28-day exposures challenged with SEB had a dose dependent reduction in activation/proliferation as measured by CD71 positivity. Cytotoxic T-cells in 0 and 200 mg/kg i.d. treatment groups were 12.8% ± 4.0% and 10.6% ± 4.4% vs 12.8% ± 4.0% and 10.6% ± 4.4% in the 90-day tungstate-exposed mice. Reductions were observed only in lymphocytes from the spleen. Alterations of activated cytotoxic/helper T-cells may result in increased susceptibility to infections. These data suggest tungstate exposure may have detrimental effects on the adaptive immune response.

329 A MULTI-SPECIES MATHEMATICAL MODEL OF THE REGIONAL DEPOSITION AND CLEARANCE OF CHEMICAL AND BIOLOGICAL AGENTS IN THE LUNG.

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Quantifying the dose delivered to the different regions of the respiratory tract via inhalation of chemical or biological agents is critical to extrapolation of animal experimental toxicity data to humans. Differences in lung morphometry, breathing patterns, and aerosol or vapor characteristics lead to different deposition patterns, and different clearance mechanisms and rates dominate in different regions of the lung. A mathematical model can be used to regionalize deposition as a function of these changing factors, and thus provide a rational basis for extrapolation of experimental toxicity data to humans. Models were developed for rabbits, guinea pigs, rats, and humans. The airways are grouped by branching generation, characterized by the average number, length, diameter, branching angle, and gravity angle of the airways. For aerosols, deposition via diffusion, sedimentation, and inertial impaction was modeled for the tracheobronchial (TB) and pulmonary (PUL) airways, and an empirical head deposition model was fit to Raabe (1975) particle deposition data. For vapors, a diffusion model estimates deposition into the mucus layer in the TB region, and blood/air partitioning is used to model the exchange with the blood in the PUL region. Clearance in the head and TB regions was via mucociliary clearance and tissue uptake. PUL region clearance of aerosols was mediated by alveolar macrophages. Monte Carlo analysis of deposition with variability distributions for breathing frequency, tidal volume, and breath pause time shows significant variation in deposition, both within and across species. Model predictions reveal significant differences in deposition patterns between those of experimental animal species and humans. The model was used to simulate the inhalation kinetics of GB vapor, predicting significant upper airway scrubbing, as well as the inhalation of aerosols of biological agents. These results demonstrate the utility of mechanistically-based in silico models, both in terms of explaining variability in results, and in extrapolation of animal toxicity data to humans.

330 RADIOPROTECTIVE EFFECTS AND MECHANISMS OF ACTION OF GENISTEIN.

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Radioprotective compounds have applications in clinical oncology, space travel, radiation site cleanup, radiological terrorism, and military scenarios. The ideal radioprotector would be non-toxic, increase survival, and would not degrade performance. In the present studies, the radioprotective and behavioral effects of the soy
isolavone genistein were investigated in adult CD2F1 male mice. Animals were ad-
ministered a single subcutaneous (SC) dose of genistein 24 h before a lethal dose
(LD90/30) of 60Co gamma radiation. There was a significant increase in 30-day survival for animals receiving genistein, with a DRF of 1.16. A DRF of 1.16 was obtained at a genistein dose (200 mg/kg SC) that did not result in any adverse pathology or performance decrement. The improvement in survival was related to accelerated neutrophil and platelet recovery, resulting from earlier and more pronounced multilineage, hematopoietic progenitor cell reconstitution in the femoral marrow compartment. Protection of the bone marrow was associated with a genistein-induced transient pause in the cell cycle where hematopoietic stem cells remained in the Go quiescent phase, the part of the cell cycle associated with specific DNA-repair mechanisms and resistance to radiation-induced DNA dam-
age. Genistein was also effective orally when given in daily gavages for two to six days before irradiation. These results demonstrate that genistein at nonlethal doses is an effective radioprotectant when administered parenterally or orally to mice.

we found that increased A-rule product formation correlated with increased reac-
tion incubation time (5-60 min) and RIP concentration (10 nM to 500 nM ricin) at 55 °C and pH 5.0 buffer. Ricin showed saturating kinetics in the substrate concent-
tration range of 2-64 μM (Km = 5.3 μM). The revised assay method should allow detection of RIP enzyme activity in samples that are incompatible with conven-
tional RNA-based RIP substrates.

Nerve agents irreversibly inhibit the enzyme acetylcholinesterase, causing a rapid accumulation of the neurotransmitter acetylcholine and an overstimulation of cholinergic effector sites in the brain and periphery. A notable sign of nerve agent poisoning is the development of status epilepticus seizures, which lead to brain damage if not rapidly controlled. The present research evaluated drugs that could be delivered as adjuncts to standard antidotes to control nerve agent-induced seizures when treatment is substantially delayed, as in a terrorist situation like the Tokyo subway incident. Rats, previously prepared with electrodes to record elec-
troencephalographic (EEG) activity, were exposed to a convulsant dose of the nerve agent soman. Seizures were allowed to develop, and the standard treatment for nerve agent intoxication—strospine, 2-PAM (an oxime cholinesterase reactivator), and diazepam (a benzodiazepine)—was delayed for 5, 10 or 20 min after seizures started. This treatment alone was unable to stop seizure activity. Drugs from various pharmacological classes (anticholinergics [scopolamine, caramiphine, procyclidine, benactyzine, pentin, G-3063]; GABA modulators [midazolam]; NMDA antago-
nists [ketamine, memantine]) were added as adjuncts to the standard treatment reg-
imen. Dose-effect curves were determined for drugs effective in stopping the seizures at the different times. Anticholinergic drugs showed a time-dependent loss of anticonvulsant potential as the treatment delay was lengthened. Of the anti-
cholinergics tested, the anticonvulsant effect of caramifpine was significantly less af-
fected by progressive treatment delays. Midazolam rapidly lost anticonvulsant effec-
tiveness with delays as short as 10 min. Ketamine produced robust anticonvulsant effects over a narrow dose range, while memantine was ineffective as an anticonvulsant at any dose tested. The results indicate that an anticholinergic drug such as caramiphine may provide substantial anticonvulsant benefit as an adjunct to stan-
dard nerve agent therapies.

In a rat model, diethylparaoxon (PO) at a dose equal to 50% of the MLD induced a decrease in respiratory rate (f) resulting from an increase in expiratory time (TE). These effects were completely but transiently reversed by a 50 mg/kg dose of pralidoxime (PRX). Methods: Male F1B6D2 mice (20 g) were used. The MLD of PO administered subcutaneously was assessed using the up-and-down method. PO-in-
duced respiratory toxicity was assessed by whole body plethysmography in awake, unrestrained animals. Pralidoxime (PRX) methylsulfate at doses of 10, 50, 100, and 150 mg/kg (PRX base) were administered intramuscularly at the maximum of PO-
induced respiratory toxicity. Statistical analysis used two-way ANOVA for repeated
measurements Results: The MLD of PO was 0.997 mg/kg. A dose of 0.5 mg/kg was used as 50% of the MLD. In comparison with controls, PO induced the rapid onset of hypothermia from 90 min after PO injection till the completion of the study, 180 min after PO injection. In comparison with controls, PO induced an in-
crease in the total time (TTot) which resulted from an increase in both inspiratory time (TI) and TE; the tidal volume (VT) increased. The minute volume was un-
changed. In comparison with PO-poisoned rats, the 10 mg/kg dose of PRX did not induce any significant effects, the 50 and 100 mg/kg dose induced a non significant decrease in TTot. The 150 mg/kg dose induced a significant and complete reversal of PO-induced increase in TTot which resulted from a decrease in both PO-in-
duced increase in TI and TE from T+45 to T+150 min after PO administration.

The 150 mg/kg dose of PRX completely reversed the effects of PO on respiratory times while there was no significant effect on PO-induced increase in VT. Conclusions: Rat and mice models of PO poisonings resulted in similar effects on f, TTot, and TE while TI was increased significantly in mice only. High dose of PRX was required to induce the complete and long-lasting reversal of respiratory toxicity of PO at a dose equal to 50% of the MLD in mice.
the use of oximes to reactivate acetylcholinesterase (AChE) after organophosphate poisoning has been under investigation for years. Recently, there has been an intense interest in developing effective non-oximes. CM-2,514 is a non-oxime that, along with a series of other novel compounds, is under evaluation as an add-on neuroprotective post-exposure therapy with the triad of antidotes, atropine, 2-PAM and diazepam (Defense Threat Reduction Agency Grant - FAB650-0506518). In addition, CM-2,514 is being evaluated as a 2-PAM replacement. CM-2,514 was evaluated in male Swiss Webster mice exposed to LD50 multiples of sarin, soman or tabun, respectively. Preliminary data indicate that CM-2,514, in comparison to 2-PAM, has equal (sarin) or improved (soman or tabun) efficacy on survival rates in mice challenged with CWAs. CM-2,514 increased survival when added to the standard therapy of atropine, 2-PAM and diazepam and substituted for 2-PAM (when withheld from the treatment). CM-2,514 matched the increased survival produced by 2-PAM when combined with atropine alone. Histological evaluation of the brain confirmed that CM-2,514 retained neurite outgrowth as well as, or in vivo reactivation of AChE, compared to that provided by 2-PAM. Safety studies with CM-2,514 in other mouse behavior and side-effect studies, such as body temperature, FOB, and spontaneous activity indicate that CM-2,514, in comparison to 2-PAM, has equal (sarin) or improved (soman or tabun) efficacy on survival rates in mice challenged with CWAs. CM-2,514 increased survival when added to the standard therapy of atropine, 2-PAM and diazepam and substituted for 2-PAM (when withheld from the treatment). CM-2,514 matched the increased survival produced by 2-PAM when combined with atropine alone. Histological evaluation of the brain confirmed that CM-2,514 retained neurite outgrowth as well as, or in vivo reactivation of AChE, compared to that provided by 2-PAM. Safety studies with CM-2,514 in other mouse behavior and side-effect studies, such as body temperature, FOB, and spontaneous activity indicate that CM-2,514, in comparison to 2-PAM, has equal (sarin) or improved (soman or tabun) efficacy on survival rates in mice challenged with CWAs.
The absorbance change per minute was converted to micromole cyt c oxidized per minute and normalized to mg mitochondrial protein. For data analysis, activity was plotted as a function of CN dose. Overall, the thiosulfate control antidote provided significant protection against the cyt c oxidase activity. The T3 treated mice showed reduced cytchrome c oxidase activity even in the absence of cyanide. In both treatment series, addition of exogenous rhodanese did not seem to significantly enhance the protection against the cyt c oxidase inhibition, but the addition of sodium nitrite did. These findings can lead to a better understanding of the protection mechanism by various cyanide antidotal systems. These studies were supported by the ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE under the auspices of the U.S. Army Research Office Scientific Services Program and the Robert A. Welch Foundation at Sam Houston State University, Huntsville TX.

**340 SHORT-TERM AND LONG-TERM DIETARY RESTRICTION MODIFY SOMAN TOXICITY IN MALE GUINEA PIGS OF DIFFERENT AGES.**

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Age and fasting have been shown separately to modify organophosphate toxicity in rodent models. This study evaluated diet restriction (ad libitum feeding vs. 80% of the recommended amount) and fasting (fed 90 minutes prior vs. not fed for 18 h prior to exposure) on survival, toxic signs, body weight, blood glucose, car-borylactase, acetylcholinesterase, and butyrylcholinesterase in male guinea pigs of different ages (60 vs. 150 days old) exposed acutely to 1.0 LD50 soman. Toxic signs scores were greater in the diet-restricted animals than in ad-lib animals. Young animals exhibited higher toxic signs scores than old animals. The diet-restriction manipulation was more successful in the young animals than in the old animals in that greater relative between-group differences in body weight were observed in the young animals (26%) than in old animals (4.8%). For ad-lib fed animals that were not fasted, survival approximated the LD50, as expected. In contrast, all other diet groups (ad-lib-fasted, diet-restricted-fasted, and diet-restricted fed) exhibited decreased survival in young animals. For old animals, only the diet-restricted-fasted group exhibited decreased survival. These data show a toxicity enhancing effect of fasting and diet restriction, particularly when combined. Age was positively related with survival, perhaps due to a decreased effect of the diet manipulations in older animals. Prevailing levels of glucose prior to exposure provided a better prediction of survival than did esterase levels, although some differences in esterase levels were found. The results of the present study indicate that age and dietary variables play an important role in the toxicity of nerve agents in guinea pigs. These factors are particularly relevant to the assessment of the neurobehavioral toxicity of nerve agents, wherein older diet-restricted-fasted guinea pigs are commonly employed.

**341 RICIN TOXICITY IN BALB/c 3 T3 CELLS: CORRELATION OF TOTAL PROTEINS WITH DOSE LEVEL BY MASS SPECTROMETRY BASED PROTEOMICS.**

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Ricin has remained a significant potential biological threat agent due in part to its wide availability and ease of extraction from the castor bean. It is a glycoprotein made up of A- and B-chains with the B-chain facilitating transport of the lectin into the cell. Once inside, it kills the cell by inhibiting protein synthesis. Ricin is considered extremely toxic to man by multiple routes of exposure. We have analyzed cellular protein extracts from BALB/c 3 T3 murine fibroblasts dosed with varying concentrations of ricin by liquid-chromatography tandem mass spectrometry (LC-MS/MS) to determine whether LC-MS/MS can be employed for toxicity independent of traditional cytotoxicity assays. Proteomics based on MS data can yield biomarker information, but model-building with IC-50/EC-50 values obtained from traditional cytotoxicity assays has been required to correlate the biomarker concentration with toxicity. Employing a method whereby no adjustment is made for cell concentration as toxin dose increases, we show that the average number of proteins identified is related linearly to the expected percent inhibition. These results indicate that, if at least eight ricin concentrations are included as is done for typical dye-based toxicity assays, toxicity can be determined and effective dose values obtained directly from LC-MS/MS. Funding received from the Defense Threat Reduction Agency.

**342 SOLVENT EFFECTS ON THE PERCUTANEOUS TOXICITY OF V AGENTS.**

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Since V agents are extremely toxic, researchers studying cutaneous applications in guinea pigs have had to either apply volumes of neat agent at the physical limits of syringes or use carrier solvents. To date no published comprehensive study has compared different solvents/concentrations used in the field and determined the effects they may have on median lethal doses (MLD) values and the rate of absorption. We determined the MLD of the nerve agents VX and VR when they were percutaneously administered to guinea pigs using the carrier solvents methylene chloride, isopropyl alcohol (IPA) and hexane in a range of concentrations. Anesthetized guinea pigs whose fur was clipped were percutaneously exposed to either VX or VR using a Hamilton syringe and mortality was measured after 24 hours. Although MLDs for VR varied between 0.21 mg/kg and 0.13 mg/kg, none of the differences were statistically significant. The MLDs for VX varied between 0.22 mg/kg and 0.12 mg/kg. Varying the concentration of the solvent did not significantly affect the MLD of VX; however, there was a statistical difference in the MLD between 90% hexane (0.22 mg/kg) and 50% IPA (0.12 mg/kg). To examine the effect of carrier solvents on the rate at which VX enters the blood stream, animals with implanted jugular catheters were exposed to 0.15 mg/kg of VX either in neat form or in a 90% IPA solution. Blood samples were collected at 1, 2, 4, 6 and 24 hours post-exposure and acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) levels were measured. Neat VX entered the blood stream significantly more rapidly than VX in IPA: all measured AChE levels reached zero by 4 hours after neat VX application but required 6 hours to reach zero when VX in IPA was applied. This data provides some support for using carrier solvents when rodents are the model but only in cases where rate of absorption in the first 24 hours post-exposure is not critical.

**343 EXPRESSION OF PROLIFERATION AND SENESCENCE MARKERS IN SULFUR MUSTARD EXPOSED MOUSE SKIN.**

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Skin exposure to chemical vesicants such as sulfur mustard generally produces more tissue damage than incisional or thermal wounds. The cells leading re-epithelialization to cover a wound are less mitotically active than the proliferating cells behind them. A time-course study using mouse ear skin exposed to a low dose (0.08 mg) of sulfur mustard resulted in a delayed onset of wound repair in comparison to that expected by other wound types. Neutrophils and macrophages were identified and their infiltration correlated to particular times. Immunohistochemical and RT-PCR studies using markers of skin injury and repair demonstrated that there was co-expression of laminin γ2 and p16 at the migrating front of healing wounds. Laminin γ2 is known to promote epithelial sheet migration over the wound bed and p16 is a protein that induces senescence in keratinocytes. In contrast the hyperproliferating cells behind the migrating front were abundant in both keratin 5, an intermediate filament protein of basal and proliferating epithelial cells and Ki67, a marker of cells in the growth stage of the cell cycle. Understanding the basic mechanism of action of vesicant-induced skin injury and repair should help to identify new targets of potential medical countermeasures against chemical vesicants. This work is supported by ES005022, EY09056, and AR055073.

**344 A TOXICOLOGICAL, BIOCHEMICAL, AND PHYSIOLOGICAL ASSESSMENT OF LUNG AND SYSTEMIC INJURY IN RATS EXPOSED TO INHALED SULFUR MUSTARD ACROSS DOSE AND TIME.**


Sulfur mustard (HD) is a blistering agent (vesicant) that causes severe chemical burns to the skin, eyes, and airways. HD was used as a chemical warfare agent (CWA) in the Iran/Iraq conflict and more than half of surviving HD-exposed casu-
alties suffer from permanent lung injuries. HD remains a serious threat with no effective antidote. Despite research in the 90 years since HD was developed as a CWA, the mechanisms and timing of the development of these pathologies are poorly defined. As a model of the mouth-breathing human, rats were intubated and ventilated for 10 min with nebulized HD or vehicle to achieve total doses of 0.5, 1.75, 2.25, and 3 mg/kg. Rats were euthanized 3 h post-exposure or maintained as long as possible (to 6 mos) until respiratory distress or weight loss necessitated their removal from the study. Rats were necropsied, blood chemistry was analyzed, and lungs were subjected to pathologic analysis. These early data show elevated pCO2 and lowered blood pH and pO2 in the 24-48 h after 3 mg/kg HD exposure. Many 3 mg/kg HD rats showed a plateau in weight gain 3 wks post-exposure that was an excellent predictor of mortality many weeks later. Rats that survived to 3 mos after 3 mg/kg HD exposure showed increased hematocrit and total hemoglobin by ~75% early on. Airway epithelial necrosis occurred as early as 24 h after 3 mg/kg HD exposure, while alveolar edema and exudate followed at 3 wks post-exposure. Lungs at 6 wks after 3 mg/kg exposure showed thickening of alveolar walls, fibrosis, inflammation, and airway blockage by neoplasia, mucus, and epithelial sloughing. White patches on the lung surface were consistent with mesothelioma, and some epithelial metaplasia demonstrated stratified squamous morphology, a prequel to sarcoma. These data provide the first long-term examination of HD-induced lung injury and systemic effects. This project was supported by Defense Threat Reduction Agency project #5.F00095.07_RC_C.

### Identification of Potential Molecular Targets for Therapeutic Countermeasures to Nerve Agent Toxicity

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The biological pathways involved in nerve agent-induced neurodegeneration are not well understood. To determine these pathways, we analyzed gene expression changes following sarin exposure. Male Sprague-Dawley rats were challenged with 1.0 x LD50 sarin, using saline as a vehicle. One minute after seizure onset, the animals were treated with atropine sulfate and 2-pyridine aldoxime methylchloride. Thirty minutes later, they were given diazepam. Control animals received an equivalent volume of vehicle and drug treatments. The pituitary, hippocampus, amygdala, and several brainstem structures were harvested at 0.25 h, 1 h, 3 h, 6 h, and 24 h post-exposure, and total RNA was processed for microarray analysis. Principal component analysis revealed the major sources of variability within each tissue group as seizure occurrence and the time at which the tissues were harvested. An analysis of variance identified genes significantly changed in all five tissues of seizing animals for each time point, and gene ontology analysis revealed inflammatory pathways, such as interleukin (IL)-6 and IL-10 signaling, as being most significant. From these analyses, tumor necrosis factor, IL-1 receptor, and c-Jun N-terminal kinase were identified as potential therapeutic targets for future development of drug therapy against nerve agent toxicity.

The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army or DoD. The experimental protocol was approved by the Animal Care and Use Committee at USAMRICD and all husbandry procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (NRC, Pub# 85-23, 1996).

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### Evaluation of a New Antidote to Chemical Warfare Agents in Mice

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Treatment of exposure to organophosphate chemical warfare agents (CWA’s) relies on a combination of agents that increases the chances of survival and blocks the cholinergic response to CWAs. Protecting the CNS from the long term neurodegeneration produced by CWA exposure is time and dose dependent and current therapy would benefit from the addition of a potent CNS protective agent. Furthermore, doses of one currently used anti-cholinergic, atropine, produce debilitating side effects, which can be toxic when given in the absence of CWAs. CM-2,550 is a clinically used drug that has both neuroprotective (multiple pharmacology) and anti-cholinergic properties. CM-2,550 is under evaluation in Swiss Webster mice as a stand-alone pre-treatment, an add-on to current therapy, and as a replacement for or add-on to lower doses of atropine. Preliminary data indicate that CM-2,550 is effective as both a pre-treatment and post-treatment when given alone against sarin. CM-2,550 also improves survival when used as a substitute for atropine in the standard treatment of atropine/2-PAM/diazepam. When added to lower doses of atropine, CM-2,550 produced greater survival than did the standard atropine dose. CM-2,550 also was as effective as atropine at blocking the tremors produced by the cholinergic agonist oxotremorine. Histological evaluation of the neuroprotective properties of CM-2,550 against sarin is in progress. These data indicate that CM-2,550 should be further evaluated as a multi-use drug for treatment of CWA exposure and as an agent to reduce or eliminate the side effects of anti-cholinergics such as atropine.

### USE of TOXICOGENOMICS and NETWORK MODELING to IDENTIFY the NRF2-MEDIATED OXIDATIVE STRESS RESPONSE PATHWAY as a MECHANISM OF ACTION for NITROAROMATIC EXPLOSIVES and PROPELLANTS

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Munitions compound TNT, its environmental degradation products 2A-DNT and 4A-DNT, and other munitions, 2,4-DNT and 2,6-DNT contaminate military firing ranges and retired ammunition plants. Although toxicity has been characterized for these compounds, little is known of their mechanism of action. Study objectives included utilizing physiological alterations, gene expression profiles, and network modeling to identify mechanism of action of one or more of the compounds. Animals received a single oral gavage of one of five doses of one of the compounds with sacrifice at 24 or 48 h. Complete blood counts, clinical pathology and histopathology of liver, and liver genomics were evaluated. Observations included sinusoid congestion in liver (2,6-DNT), vacuolation in liver (2,6-DNT), and reduced the level of the collagen XVII clipping enzyme, TACE. These data suggest that the adverse corneal effects of vesicants can be ameliorated by Amino Plex. Supported by FY000056 and AR055073.
Targrs shared by these compounds in rats and 2,4-DNT in fish include lipid metabolism, PPARs, and HNF transcription factor suggestive of a common mechanism of action across species. These data indicate a toxic mode of action of nitrosor- natomics in distantly related species. In conclusion, we found TNT and the DNTs have some commonality in their mechanism of toxicity through the use of both physiological and genomic evaluation.

**349 MODELING ORAL ADMINISTRATION OF ORGANOPHOSPHORUS HYDROLYZING ENZYME (OPH) AGAINST PARAXON INTOXICATION.**

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Organophosphorus (OP) molecules such as paraxon, the toxic metabolite of the commonly used insecticide parathion, as well as the chemical warfare agents sarin and soman, exhibit their toxic effects by irreversible inhibiting acetylcholinesterase. One of the perspective treatments of OP poisoning is the administration of exoge- nous OP hydrolizing phosphotriesterase (OPH). Earlier studies indicated the effecti- veness of the intravenously administered OPH against paraxon intoxication em- ployed in combination with 2-PAM and atropine. Present studies are focused on developing oral OPH formulations, and in vitro and in vivo modeling for the oral OPH application. Lyophilized OPH was packed into gelatin mini-capsules, fol- lowed by a coating with various polymers, e. g. cellulose acetate-hydrogen phthalate, to make the capsules acid resistant and protecting the enclosed enzyme in the stom- ach. At the intestinal pH of 7-8 the coating is dissolved and the capsule re- leases the enzyme, making it ready for the intestinal absorption. Ability of mini- capsules to release OPH was studied by incubating them in 0.1N HCl at pH 1.2 (imitation of gastric juice) and in the mixture of citric acid and Na2HPO4 at pH=7.0 - 8.0 (imitation of intestinal media) at 37 °C for 1-3 h. OPH activity was assayed after incubation with paraxon, and the production of paranitrophenol determined spectrophotometrically. Mini-capsules were stable at pH 1.2 and re- leased the enzyme at pH 7.0-8.0. The same mini-capsules are employed in the in vivo animal studies in mice-, and guinea pig- animal models when the paraaxon is administered ip and the capsules are delivered to the stomach by using a bulging gun. These experiments are providing preliminary data for future antitodal studies against paraxon intoxication. This work was funded by NIH (Grant #: 5 U01 NS058015-02), and the Robert A. Welch Foundation (s-0011) at Sam Houston State University, Huntsville TX.

**350 EXPOSURE TO SULFUR MUSTARD INDUCES THE EXPRESSION OF INFLAMMATORY PROTEINS IN RAT LUNGS.**

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Sulfur mustard (SM), 2 (bis-chloroethyl) sulfide is a vesicant that causes severe damage to the respiratory system. The toxicity of SM is attributed to its ability to alkylate a variety of macromolecules including proteins, lipids, and nucleic acids. This can induce oxidative stress and inflammation. The role of inflammatory mediators in the pathogenic response to SM is unknown and represents the focus of these studies. Spontaneously breathing male rats (Crl:CD SD BR) were anes- thetized, intratracheally intubated and exposed to 0.7, 1.0 or 1.4 mg/kg SM by vapor inhalation. Animals were sacrificed 6 hr, 24 hr, 48 hr or 7 d post-exposure and bronchoalveolar lavage fluid (BAL) and lung tissue collected. Exposure of rats to SM caused an increase in BAL protein which was evident after 24 hr and persisted for 7 d. This was associated with increased expression of caspase-9 and cleaved caspase-3 in bronchial epithelium, markers of apoptosis. Sulfactant protein-D (SP-D), a pulmonary collectin known to protect airway surfaces and down regulate inflam- mation, decreased in the lung after SM exposure. This was correlated with thickening of alveolar septal walls, inflammatory cell infiltration into the alveoli and in- creased expression of tumor necrosis factor-α and matrix metallproteinase-9, inflammatory mediators implicated in matrix degradation and tissue injury. Expression of inducible nitric oxide synthase and cyclooxygenase-2, enzymes medi- ating production of pro-inflammatory reactive nitrogen species and prostaglandins, respectively, was also evident in the lung after SM exposure. These findings demon- strate that SM-induced lung toxicity is associated with the generation of a number of cytotoxic proteins which may contribute to the pulmonary toxicity of this vesi- ciant. Supported by NIH Grants GM034330, ES004738, CA152624, AR055073 and ES005022.

**351 DRUG EFFICACY EVALUATIONS FOR TREATMENT OF SULFUR MUSTARD OCULAR AND DERMAL INJURY.**


Sulfur Mustard (SM) is a vesicant (blistering agent) that targets lung, skin, eye, and testes. The purpose of these studies was to screen currently existing therapeutics, based on biological activity against known or hypothesized SM mechanisms of ac- tion, against SM-induced dermal and ocular injury. Agents tested for efficacy against ocular injury included doxycycline (novo formulation and soluble formula- tion; 2 mg/mL; matrix metalloproteinase inhibitor), epigallocatechin gallate (EGCG; 10 mg/mL; antioxidant); and ilomastat (0.08 mg/mL; matrix metallopro- teinase inhibitor). All agents were administered as eyedrops, 4 times/day. Agents tested for efficacy against dermal injury included doxycycline (topically as 3% gel), EGCG (50 mg/kg ip, twice daily); U0126 (20 mg/kg; subcutaneously; twice daily; MAP kinase inhibitor), simvastatin (anti-inflammatory; 10 mg/kg, 20 mg/kg, and 50 mg/kg, subcutaneously, twice daily), and aloe vera gel (99% gel, once daily). In cases where drugs were administered to guinea pigs by injection, SM exposed sites were treated daily with propylene glycol/carboxymethylcellulose gel. The right eye of each rabbit was exposed to 500 mg/m3 SM vapor for 8 minutes, and guinea pigs were exposed to 500 mg/m3 SM for 12 minutes on three, 1 cm diameter sites to in- duce lesions. Drug treatments began within 0.5 hr after exposure. Rabbits and guinea pigs were followed for 7 and 5 days post SM exposure, respectively. Eyes were evaluated daily for edema, erythema, corneal opacity, adverse effects, loss of iridal mobility, and changes in corneal clarity. Skin tissues were examined at necropsy, and histopathological changes scored. Only ilomastat showed efficacy against ocular le- sions. To date, none of the above agents were effective in reducing dermal lesions beyond changes provided by the propylene glycol gel. Verification of the efficacy of ilomastat is in progress. Research conducted under U54 NS058185-01.

**352 OXIDATIVE DNA DAMAGING EFFECTS OF SULFUR MUSTARD ANALOG CEE3 ON MOUSE SKIN CELLS.**


Sulfur mustard (HD), a chemical warfare agent, causes severe skin damage with vesication linked to the death of keratinocytes in the basal layer of the skin. DNA damage is reported to be the major cause of HD-induced cytotoxicity, which could result either via allylation or oxidative stress related mechanisms. To understand the mechanisms of these cytotoxic effects, in our recent studies employing J8F1 mouse epidermal cells and SKH1 hairless mouse skin fibroblasts, we have identified and established the cytotoxicity related biomarkers using 2-chloroethyl ethyl sulfide (CEES), a less toxic analog of HD. These studies showed an increase in phosphorylation of H2AX ser139 and p53 ser15 by 0.5mM CEE3 indicating its DNA damaging effect which was quantified employing comet assay. To assess and define the role of oxidative stress in CEE3-induced DNA damage, here we measured 8-hydroxydeoxyguanosine (8-OHdG), a ubiquitous marker of oxidative stress, and observed an increase in its levels with CEE3 treatment. Our studies in these cells also found a drastic decrease in DNA damage, measured through comet assay, with 10mM GSH 30 min pre or post CEE3 treatment, further suggesting the role of oxidative stress in CEE3-induced DNA damage. Employing JB6 cells, we next analyzed the reactive oxygen species (ROS) involved in the CEE3-induced oxida- tive stress, using MitroSOX red and dihydrothiodye dyes to measure mitochondr- onal and cellular superoxide production, respectively. Results from these experiments demonstrated an increase in superoxide production with CEE3 treatment, which was maximum at 4 h and a decline was observed by 24 h of its treatment. Further studies are underway to assess other ROS and/or reactive nitrogen species (RNS) involved in CEE3-induced oxidative stress. These studies will further assist in un- derstanding the role of oxidative and nitrosative stress in CEE3-induced skin in- jury, which could help in identifying effective antioxidant therapeutic interventions for HD-induced skin toxicity.

**353 DEVELOPMENT OF EFFICIENT AND APPLICABLE MALE SKH-1 HAIRLESS MOUSE SKIN TOXICITY MODEL WITH SULFUR MUSTARD ANALOG.**


Currently no treatments are available for the blistering, inflammatory skin lesions that appear hours after contact with sulfur mustard. For this reason, additional studies of this process, using relevant animal models, are needed. Our recent stud-
ies have established inflammatory biomarkers of HD analog, 2-chloroethyl ethyl sulfide (CEES)-induced skin injury in a model using female SKH-1 hairless mice. To further assess any sex related variations in CEES-induced inflammatory responses and skin injury, we expanded our study in to male SKH-1 hairless mice. Using effective doses of 1 and 2 mg CEES as indicated by our studies in female SKH-1 mice for 9-48h, studies in male mice demonstrated comparable changes in the inflammatory end points. For example, similar to female SKH-1 mice, CEES topical treatment to male mice induced edema seen as an increase in skin bi-fold thickness and skin wet/dry weight ratio, increases in epidermal thickness, apoptotic cell death, cell proliferation, and an earlier infiltration of inflammatory cells like macrophages, mast cells and neutrophils. Though we have established useful quantitative inflammatory biomarkers of CEES-induced skin injury in this model, we did not observe micro-vesication, an important effect of HD reported in various other animal models. Accordingly, we further increased the dose of CEES and found that exposure of male SKH-1 hairless mice to 4 mg CEES causes epidermo-dermal separation in H&E stained CEES-treated skin tissue sections indicating mico-vesication. Since vesication, as evidenced by dermal-epidermal separation, is a key component of HD toxicity to the skin, this newly developed model provides an even more relevant context for developing effective countermeasures.

Using effective doses of 1 and 2 mg CEES as indicated by our studies in female SKH-1 mice, we further increased the dose of CEES to 4 mg CEES and found that exposure of male SKH-1 hairless mice to 4 mg CEES causes epidermo-dermal separation in H&E stained CEES-treated skin tissue sections indicating micro-vesication. Since vesication, as evidenced by dermal-epidermal separation, is a key component of HD toxicity to the skin, this newly developed model provides an even more relevant context for developing effective countermeasures.

Nerve agents are not considered to be genotoxic or carcinogenic since their potent effects on both cholinesterase and cholinergic pathways are recognized and responsible for their lethality. However, studies of chronic low-dose exposure of the potent nerve agent soman (GD) in guinea pigs have shown DNA fragmentation in isolated blood leukocytes by using the single cell electrophoresis assay known as the Comet assay. We questioned whether the sensitive Comet assay could uncover DNA fragmentation in human cells that were exposed to both GD and VX under in vitro conditions. Normal strains of human small airway epithelial cells (SAEC) and normal human epidermal keratinocytes (NHEK) in culture were exposed to both GD and VX at concentrations from 0.1 μM to 50 μM for 2 h. The agents were removed by vacuum aspiration and the cells were washed a minimum of 5 times with PBS to remove any trace agent. The cells were refed with fresh media and then incubated for an additional 24 h at 37°C in 5% CO2. The cells were harvested using conventional trypsin/EDTA procedures and processed for genotoxicity analysis by the Comet assay and cytotoxicity by propidium iodide uptake via flow cytometry. After staining with SYBR™ Green (a fluorescent dye), a minimum of 50 cells for each VX and GD concentration was analyzed by fluorescence mi- croscopy using Loa’s Comet analysis software. Results from these studies show no statistical change in Comet moment (a measure of DNA damage) in GD- or VX-exposed SAEC and NHEK compared to the unexposed controls. Cytotoxicity of GD and VX-exposed cells, determined by flow cytometry using propidium iodide uptake, also showed no statistical change from controls. It is concluded that GD and VX have no direct effects on genotoxicity or cytotoxicity in SAEC or NHEK under these acute exposure conditions.

POLYMORPHISMS OF GENES INVOLVED IN THE METABOLIC ACTIVATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) CYP1A1, CYP1B1, AKR1A1, AKR1C1 AND GSTM1 IN A MEXICANS POPULATION.

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PAHs are ubiquitous environmental pollutants generated from tobacco combustion among other sources. These compounds are human carcinogens that require metabolic activation to exert their deleterious effects. However, such activation may depend on individual susceptibility and genetic polymorphisms originated from ethnic differences. Polymorphisms of human CYP1A1, CYP1B1, AKR1A1, AKR1C1 and GSTM1 are implicated in the development of cancer in populations exposed to PAHs. Thus, the frequency of CYP1A1 (Ile462Val), CYP1B1 (Val432Leu), AKR1A1 (Glu186), AKR1A1 (Asp52Ser), AKR1C3 (Gln5His) and GSTM1 (null) polymorphisms were investigated. DNA samples from 140 subjects were analyzed and gene polymorphisms were determined by Real Time PCR, and GSTM1 was assessed by PCR. The differences of the gene allelic frequencies found in this study will be compared with those of other populations and the toxicologi- cal relevance of these findings is also under assessment.

Our goal in this study was to measure the allele frequency for key pharmacogenetic loci in members of the CSKT population. These polymorphisms confer differential and potentially harmful responses in patients through altered forms of drug metabo- lizing enzymes, drug transporters, or drug targets. However, there was little baseline knowledge of the relative abundance of these variant alleles in American Indian tribes. Thus, we sought to establish genetic allele frequency for the following ten loci: CYP2D6 *3, CYP2D6 *4, CYP2D6 *5, CYP2C9, CYP2C19*2, CYP2C19*3, CYP3A5*3, CYP3A5*6 and NQO1*2 in 122 Confederated Salish & Kootenai tribal (CSKT) individuals. All participants were individually and group consented prior to study. Four of the loci had variant allele frequencies below 1% and were not analyzed further. The allele frequencies for the remaining loci were compared to published frequencies in vari- ous ethnic populations. Variant allele frequencies were also correlated with tribal blood quanta, gender, cancer incidence, and were analyzed for Hardy-Weinberg equilibrium. Allele frequencies were as follows: Caucasian/CSKT population: 22.5% blood quanta: CYP2D6*4 = 20/25; CYP2C19*2 = 13/18; CYP2C19*2 = 14/05; CYP2C9*3 = 11/06; CYP3A5*3 = 85/94; NQO1*2 = 16/32. Several of these differences approach significance with the current sample size. Additional samples are currently being analyzed. These finding could have profound implications in the safe use of etoposide, tamoxifen, cyclofosfamide, oxime-based drugs, vin- cristine, docetaxel, ritoxanec, or ifosfamide in this population.

GENETIC VARIATION IN ABCB1 AND THE SUSCEPTIBILITY TO PARKINSON’S DISEASE.

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The ABCB1 gene (or MDR1) encodes the eflux transporter P-glycoprotein (P-gp). P-gp is expressed in many tissues including the blood brain barrier (BBB) where it protects the brain from toxic substances. P-gp has been proposed to play a role in...
the susceptibility to Parkinson's disease, possibly by altering accumulation of environmental neurotoxicants in the brain. However, evidence is missing as to whether these neurotoxicants are P-gp substrates and if P-gp alters their ability to cross the BBB. Our goal is to measure P-gp transport of neurotoxicants and determine if ABCB1 genetic variation changes transport across the BBB. Our initial studies focused on the neurotoxicant paraquat. We utilized two cell lines: MES-SA cells that express no P-gp and MES-SA-DX5 cells that overexpress P-gp. In these cells we measured intracellular paraquat accumulation by HPLC detection and paraquat-induced cytotoxicity. After incubation with 500 μM paraquat, we observed higher intracellular paraquat accumulation in MES-SA cells compared to MES-SA-DX5 cells (18.2±1.3 and 1.22±0.31 pmol/mL×106 cells/hr, respectively; p=0.0001) indicating that P-gp is mediating the transport of paraquat. To confirm this, we utilized a P-gp inhibitor (GF120918) and observed a significant increase in paraquat accumulation in MES-SA-DX5 cells. We observed an approximately 10-fold increase in resistance to paraquat cytotoxicity in MES-SA-DX5 cells compared to MES-SA cells, providing further evidence that paraquat is a P-gp substrate. Alteration in P-gp transport due to ABCB1 genetic variation has been proposed to play a role in the development of Parkinson's disease. Therefore, our future studies will examine how ABCB1 genetic variation transport utilizing a lentivirus expression system in LLC-PK1 epithelial cells. This study will provide the missing data to measure P-gp-mediated transport of neurotoxicants involved in the susceptibility to Parkinson's disease and the influence of ABCB1 genetic variation.

359 STYRENE INDUCED HEALTH EFFECTS RELATED TO ALDH2 POLYMORPHISMS IN CHINESE WORKERS.

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Styrene is an important industrial chemical used worldwide. Styrene-exposed workers have been studied extensively for the induction of various types of genotoxic effects in relation to genetic polymorphisms, and the results remained conflicting. One important cause is that most of the studies performed thus far included only relatively small subjects, making it more difficult to detect possible associations and possibly suggesting false associations. To further clarify genetic modification of DNA damage levels following exposed to styrene, we recruited a relatively larger sample (more than 400 workers including styrene-exposed and non-exposed control subjects). In addition, we also evaluated the effect of aldehyde dehydrogenase 2 (ALDH2) gene polymorphisms on styrene-induced DNA damage. Owing to that not only ALDH2 is involved in the metabolism of styrene, but also approximately 40% of the population in East Asia lacks the enzyme activity due to mutant alleles of ALDH2 gene. DNA damage was measured by the FPG-modified comet assay. ALDH2 genotypes were determined by PCR-RFLP. Styrene exposure levels were estimated by the urinary concentrations of its metabolites (mandelic acid and phenylglyoxylic acid). We found no effect of styrene exposure on red blood cell count, but the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were significantly higher in styrene-exposed workers, and these effects were found in both genders. Other detailed results will be presented. To our knowledge, this is the first investigation that ALDH2 polymorphisms may have an effect on DNA damage in leukocytes caused by styrene.

360 INDIVIDUAL VARIATION IN PARAOXONASE 1 ACTIVITY IN HUMAN SERUM OVER TIME.

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Paraoxonase 1 (PON1) is a serum glycoprotein capable of hydrolyzing many pesticides. There is a substantial variation among individuals within a population due to genetic and environmental factors, including the Q192R polymorphism, which affects the efficiency of substrate hydrolysis. Low PON1 activity levels have been associated with increased risk for atherosclerosis and other disorders. It has been shown that PON1 levels are lower in older populations, so this study has measured serum PON1 activity levels in the same individuals over time. A prospective study is underway to determine if individual serum PON1 levels vary significantly over a time span of about 15 years and which changes in lifestyle, diet, or occupational exposure factors may influence PON1 levels. In a pilot study, 30 rural lowan males ages 44-73 as the study outset, about half farmers, were evaluated. Serum samples had been taken since 1994 roughly every 5 years and stored at -80°C, making three samples available from each individual. PON1 activity and Q192R phenotype were determined using phenyl acetate and CMPA (4-Chloromethyl)phenylacetate as substrates. Based on the assay results, all three phenotypes are represented in the cohort. With phenyl acetate and CMPA, 53% and 43% of subjects, respectively, did not show the expected decreased PON1 levels with age. This difference in substrate sensitivity may be due to the lower enzyme specificity for phenyl acetate. This pilot study did not conclusively confirm the assumed age-related decrease in PON1 levels at the individual level. Increasing the number of participants and including dietary, health, and pesticide exposure data from the accompanying questionnaires of this ongoing large population study will allow us to identify genetic and environmental risk factors for low PON1 status, the role of pesticide exposure, and allow us to counsel the most at-risk subgroup about their individual pesticide vulnerability. (NIEHS P42ES051661 and U07/CCU706145 from CDC/NIOSH)

361 MODULATION OF GENETIC DAMAGE AND DNA REPAIR CAPACITY BY GENETIC VARIATIONS IN THE NUCLEOTIDE EXCISION REPAIR GENE XPC.

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Xeroderma pigmentosum complementation group C protein, encoded by the XPC gene, plays a key role in DNA nucleotide excision repair (NER). XPC is highly polymorphic, with over 90 single nucleotide polymorphisms (SNPs). A few were evaluated as potential modifiers of cancer risk, however, a comprehensive evaluation of all common XPC SNPs on DNA damage-response and DNA repair capacity (DRC) has not been performed. We constructed a comprehensive haplotype map containing all XPC SNPs, analyzed the relationship between DNA damage associated with smoking, using chromosome aberrations (CA) as a biomarker, and these haplotypes. We identified 21 haplotypes that segregated into 6 phylogenetic haplotype groups (PGHAs). Our data indicate significant interaction between PGH-C and smoking for baseline CA (P=0.046). Using the mutagen-sensitivity assay (a biomarker that serves as an intermediate phenotype for cancer risk), we also observed significant interaction between smoking and PGH-D (P=0.023) and PGH-F (P=0.007) for mutagen-induced CA. To provide mechanistic explanations to our findings, we exposed human lymphoblastoid cells, with different XPC haplotypes, to UV radiation, which generates classical NER substrates (cyclobutane pyrimidine dimers and 6-4 photoproducts). Using the UVDE FLARE assay, which quantitates UV-induced DNA damage, we determined the relationship between XPC haplotypes and DRC. We hypothesized that if XPC haplotypes have functional effects, there would be a correlation between these haplotypes and DRC, and levels of UV-induced DNA damage. Our preliminary results suggest a relationship between XPC haplotypes and DRC, and provide initial mechanistic explanations to the biological effects we observed in smokers and to the reported association between XPC SNPs and cancer risk.

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Many variations in human susceptibility to chemically-induced toxicities are linked to alterations in phenotype/genotype. Such genetic diversity may influence the disposition/metabolism of a chemical. Genetically diverse murine strains were selected from the NTP-Perlegen Mouse Genome Resequencing Project (Tier 1) to examine differences in systemic exposure and/or metabolic formation and clearance following a single benzene exposure. Using 18 strains of male mice, genetic diversity appears to alter the disposition of benzene and/or its metabolites as assessed by the AUC, Cmax or Tmax of [14C] benzene equivalents in whole-blood. Current studies have focused on searching for corresponding differences in female mice from these same strains. Female mice (5 per time point) were administered a single oral dose of [14C] benzene, (0.1 mg/kg, 75 μCi/g). Blood and urine (from bladder) were obtained at 5-120 min post dose for analysis of total [14C] content. The AUC, Cmax and Tmax of [14C] equivalents in blood ranged from 34 to 119 min×μmol/mL, 0.2-6.0 μmol-equ/mL and 11-38 min, respectively. Small but notable differences were observed in the qualitative profile of benzene metabolites present in bladder urine among the various mouse strains. These results indicate the inter-strain differences in benzene metabolism and disposition are less variable in females than those seen in males of the same strains. Based on these results, strains showing notable differences in these parameters will be selected for detailed pharmacokinetic/metabolic studies. This research was supported in part by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Science Center Grant Number P3-ES-06694.
I. R. DILI is "idiosyncratic," meaning the drug is safe for the vast majority of treated patients. The most problematic and poorly predictable form of drug-induced liver injury (DILI) is the major adverse drug event that leads to regimentation of the functional depression, aplastic anemia, and hematological cancers. Benzene exposure is known to perturb the normal maturation of pluripotent cells in the functional hematopoietic system of bone marrow. Previously, we reported the differences in the PK of total 14C-radioactivity in femoral bone marrow following a single oral 14C-benzene administration over time in males or females from selected strains. Mice were administered a single oral dose of 14C-benzene (0.1 mg/kg, 75 µCi/kg) and euthanized at t = 5-2min-2. Femurs were extracted, bone marrow was extruded, total protein content was determined, and total 14C content was quantified using LSC. Pharmacokinetic parameters were estimated using a 1-compartment model assuming 1st-order kinetics. The AUC, Cmax and T max of 14C-radioactivity were determined and used as a basis for differential analysis of the PK. This research was supported in part by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Science Center Grant Number P3-ES-06694.

Dose, disposition and metabolism of a compound are known to affect host susceptibility to toxicity and carcinogenesis. Benzene exposure is linked with immunologic depression, aplastic anemia, and hematological cancers. Benzene exposure is known to perturb the normal maturation of pluripotent cells in the functional hematopoietic system of bone marrow. Previously, we reported the differences in the PK of total 14C-radioactivity in femoral bone marrow following a single oral 14C-benzene administration over time in males or females from selected strains. Mice were administered a single oral dose of 14C-benzene (0.1 mg/kg, 75 µCi/kg) and euthanized at t = 5-2min-2. Femurs were extracted, bone marrow was extruded, total protein content was determined, and total 14C content was quantified using LSC. Pharmacokinetic parameters were estimated using a 1-compartment model assuming 1st-order kinetics. The AUC, Cmax and T max of 14C-radioactivity were determined and used as a basis for differential analysis of the PK. This research was supported in part by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Science Center Grant Number P3-ES-06694.

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USE OF A GENETICALLY DIVERSE PANEL OF CELL LINES TO IMPROVE PREDICTIVE POWER OF IN VITRO SAFETY SCREENS.

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Most studies assessing the pulmonary toxicity of nanoparticles are done by instillation of a dispersion of nanoparticles, rather than by inhalation of an aerosol containing nanoparticles. To gain insight in the importance of the exposure route, rats were exposed by nose-only inhalation to 4.1 (± 1.1) g/m3 or 27.0 (± 4.3) g/m3 aggregated silicon dioxide nanoparticles or to clean air for 5 days, 6 hours/day and in parallel a smaller dose to rats by intratracheal installation of 5.7 ± 0.3 µg of aggregated silicon dioxide nanoparticles. The size of the particles was determined using a laser diffraction analyser. The results of the experiments have shown that the exposure route is of importance for the development of pulmonary toxicity. The inhalation route caused more severe effects than the instillation route. The in vivo results suggest the importance of the exposure route for the development of pulmonary toxicity.

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PS 364 NMR AND MICROARRAY BASED ANALYSIS OF MOUSE LIVER FOLLOWING EXPOSURE TO TRICHLOROETHYLENE.

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Trichloroethylene (TCE) is a widely used industrial chemical, and a common environmental contaminant and it is a well-known carcinogen in rodents and a probable carcinogen in humans. Studies utilizing panels of mouse inbred strains afford a unique opportunity to understand both genetic and environmental influences in responses to TCE. We tested the hypothesis that individual and liver-specific toxic effects of TCE are genetically controlled and that the mechanisms of toxicity and susceptibility can be uncovered by exploring responses to TCE using a diverse panel of inbred mouse strains. TCE (2100 mg/kg) or corn oil vehicle were administered by gavage to 6-8 wk old male mice of 15 inbred strains. Serum and liver were collected at 2, 8, and 24 hr post dosing and were analyzed for markers of hepatocellular injury, gene expression and metabolomic profiles. NMR-based metabolite profiling was performed on liver extracts at each time point and gene expression assessed in liver at 24 hrs. At each time point a subset of mouse strains showed distinct metabolic perturbations due to the TCE treatment. Global metabolomic analyses were carried out to determine the critical perturbations in the TCE susceptible mouse strains. Differences in glycolytic and mitochondrial precursors were observed between strains. Gene expression data showed that 5 out of 15 TCE susceptible mouse strains. Differences in glycolytic and mitochondrial precursors were observed between strains. Gene expression data showed that 5 out of 15 TCE susceptible mouse strains.
treated by instillation also showed increases of total cells, viable cells and neutrophils in both concentration groups. After two decades of intensive research, however, its potential cytotoxicity for predicting in vivo health outcomes. ENP surface chemistry, aggregation state, and solubility may influence their ability to modulate immune defense and inflammatory responses in organisms. Specifically, ENPs may be recognized by Toll-Like Receptors (TLRs), triggering an immune response through the Nuclear Factor-kappa B (NF-kB) transcription factor. To test the ability of ENPs in activating this pathway, Human Embryonic Kidney cells (HEK293) stably expressing TLRs 2, 4, and 5 were transfected with the NF-kB reporter gene. The ability of select ENPs to transcriptionally activate NF-kB were determined using a Luciferase assay. Exposure of HEK293-TLR2 expressing cells to non-toxic doses of Cu nanoparticles in mice produces inflammatory responses, oxidative stress and alters host defense mechanisms. Our hypothesis is that inhalation sub-acute exposure of Cu nanoparticles in mice produces inflammatory responses, oxidative stress and alters host defense mechanisms. This research may also provide insight into the role of ENPs in inflammatory mechanisms.
Currently there is no standardized method for making nanomaterials or evaluating their toxicity. Studies are showing great variability and lack of reproducibility in results, with the discrepancies partially as a result of the assays used to evaluate exposure toxicity. While the traditional biochemical methods for evaluating toxicity have been employed there is an issue of how the nanomaterials themselves interact with the dyes. For example, the MTS assay is read at a wavelength of 490nm and this wavelength corresponds to the spectral signature for silver nanomaterials and therefore can give false stimulatory effects. The current methodologies need to be evaluated to determine the toxicity of the nanomaterials and ensure there is no interaction between the nanomaterials and the reagents used to complete the assays. This current study evaluated mechanisms of nanomaterial toxicity using mitochondrial function, loss of mitochondrial membrane potential, and formation of reactive oxygen species as indicators of toxicity, and compared the data obtained on the BD Pathway 435 confocal microscope with HCA analysis with data obtained from a BioTek plate reader. Furthermore, nanoparticle uptake and localization was evaluated using the 3D imaging capabilities on the BD Pathway 435. These studies demonstrated that the in situ characteristics of nanoparticles are critical to the potential toxicity.

### Metal Oxides Influence Cellular Homeostasis via Multiple Interconnected Signaling Pathways.

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Influence of cellular homeostasis was studied using various nanoparticles (NPs) of metal oxides. NPs induced oxidative stress (OS). An inverse correlation between OS and cell viability was observed (p < 0.05). Biochemical analyses and gene expression studies revealed that OS leads to cell death via DNA injury, membrane depolarization, loss of membrane integrity, and induction of death-related genes. NPs elevated intracellular calcium levels ([Ca²⁺]i) in a time- and concentration-dependent fashion. An inverse relation between [Ca²⁺]i and cell viability was identified (p < 0.05). [Ca²⁺]i can be partially attenuated by antioxidants N-acetylcycteine indicating a link between calcium modulation and OS. Nifedipine partially attenuated the increase in [Ca²⁺]i suggesting a contribution of L-type calcium channels. Direct membrane lipid peroxidation resulting in leaky cytoplasmic membrane and the release of calcium from intracellular stores may participate in calcium influx as well. Initiation of OS and intracellular calcium modulation via multiple mechanisms is discussed. Finally, a diagram depicts relationships between NPs, OS, [Ca²⁺]i and cell death.

### Effects of Quantum Dots on Cellular Stress Markers in HepG2 Cells.

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Quantum dots (Qdots) are nanometer-sized crystals with photochemical and photophysical properties that offer distinct advantages over organic dyes and fluorescent proteins, such as high levels of brightness and photostability. Qdots are generated as a core size and coating combination. The core of the Qdot is made up of cadmium, sulfur, and selenium. While metals utilized for this core composition are known to be toxic, very little is known regarding potential toxicities elicited by various surface coatings. Previously, we found that poly(malic anhydride-alt-1-tetradecene), tri-n-octylphosphineoxide (PMAT-TOPO) co-polymer CdSe Qdots appear to colocalize with cultured HepG2 cells in a dose-dependent manner over a period of 24 hours. In addition to Qdot uptake, our preliminary results indicate that this particular class of stable Qdots has no adverse effects on HepG2 cells under the conditions assessed with the MTT cytotoxicity assay. To further assess for more sensitive markers of cellular stress, we measured various endpoints (uptake, glutathione content and mitochondrial membrane potential) by flow cytometric analysis after 24 hours of exposure to Qdots. Consistent with our previous studies, measurements by flow cytometry indicate a dose-dependent increase in uptake. Next, we measured for changes in mitochondrial membrane potential with nonsodium chloride orange. No changes in membrane potential were evident over the range of concentrations tested. Interestingly, levels of glutathione appear to be slightly reduced at higher doses of Qdots with PMAT-TOPO coating. Work is currently ongoing to more thoroughly characterize effects on cellular functioning elicited by Qdots. This work was supported by NIEHS grants 1R01ES016819, P30ES07033 and T32ES07032.

### Comparative Toxicity of Lunar, Martian, and Earth Dusts in Human Skin Fibroblast Cells.

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NASA has plans to build a permanent structure on the Moon by 2020; therefore they are concerned about the potential toxicity of lunar dust. NASA also hopes to visit Mars, and thus are also concerned about Martian dust. In our study we compared the cytotoxic and genotoxic effects of two lunar dust simulants (JSC-1AVF, JSC-1AF) to Earth dust (urban particulate matter), and a Mars dust simulant (MARS-1AF) in human skin fibroblast cells (BJ/TE17). Comparing the cytotoxicity of these chemicals shows that the Mars dust simulant is the most cytotoxic after a 24 h and a 120 h. The JSC-1AVF lunar dust simulant is more cytotoxic than the JSC-1AF, which is possibly due to the AVP particles being smaller. The cytotoxicity of Earth dust is similar to Mars dust. The 120 h genotoxicity shows that all four simulants show similar effects. This work is supported by the Maine Center for Toxicology and Environmental Health, NASA grant EP-08-01, and the Maine Space Grant Consortium.

### Effects of Fentanyl Citrate on Fertility and Early Embryonic Development to Implantation in Rats.

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The objective of this study was to determine the fertility effects of fentanyl citrate in male and female rats. Four treatment groups of 25 male and 25 female ([Crl:CD® (SD)] rats, received Fentanyl Citrate subcutaneously at respective dose levels of 12.5, 25, 50 (increased to 100 in females on day 8), and 300 μg/kg/day. Two groups of 25 animals per sex served as controls and received the vehicle, 0.9% Sodium Chloride for Injection, USP. Six additional groups of 25/sex were untreated and utilized for breeding with each assigned treatment group. An additional high dose of 300 μg/kg/day was later added in the study at the request of a regulatory agency. Males and females were treated for 28 and 14 days prior to mating, respectively. Dosing continued in males through mating and post-mating: females were dosed through gestational day 7. Mortality was observed at 300 μg/kg/day in male and female rats and at 50/100 μg/kg/day in female rats. Exaggerated pharmacological effects including decreased activity, prostration, loss of righting reflex and slow breathing were observed at doses >25μg/kg/day. Decrements in body weight parameters, and food consumption were observed at 50/100 and 300 μg/kg/day. Sexual and accessory sexual organ weights were significantly decreased at 300 μg/kg/day in comparison to the controls. Fertility and fecundity indices were significantly reduced in naïve females mated with males receiving 300μg/kg/day. Likewise, uterine weights were significantly reduced in naïve females mated with males at 300 μg/kg/day. The uterine weight reduction correlated with a significant decrease in implantation sites and viable embryos, and with increased post-implantation loss. Based on the results of this study, the NOAEL for general toxicity was 12.5μg/kg/day for both sexes. The NOAEL for reproductive toxicity in treated male and female rats were 50/100μg/kg/day and 300μg/kg/day, respectively.

### Evaluation for Ovarian Toxicity of Sodium Valproate Using Rat Cultured Ovarian Follicles.


Sodium valproate (VPA), an anisiepileptic drug, is known to induce endocrine side effects including characteristic symptoms of poly cystic ovary syndrome in women with epilepsy, and cystic follicles in the ovaries have been reported in VPA-treated
rats. In this study, we evaluated the effects of VPA on the follicular development and synthesis of ovarian steroid hormones using rat cultured ovarian follicles. Ovarian follicles were sampled from the ovaries in female Sprague-Dawley rats (14 days old) and cultured for 48 hours with VPA (0, 0.2, 1.0 and 5.0 mM) following 24-hour pre-culture. At 0, 24 and 48 hours of the VPA treatment period, follicle diameters were measured. At 48 hours of the VPA treatment period, viability of granulosa cells (GCs) was assessed by trypan blue staining, and progesterone, androstenedione, testosterone and estradiol levels in the culture media were assayed using a commercial ELA or ELISA kit. To evaluate of VPA on the synthetic pathway from progesterone to estradiol, the progesterone androstenedione, androstenedione/testosterone and testosterone/estradiol ratios were calculated. At 5.0 mM, follicular development was suppressed with low viability of GCs. Androstenedione, testosterone and estradiol levels were lower at 0.2 mM and above, whereas a progesterone level was higher at 5.0 mM, than the control. The progesterone/androstenedione and androstenedione/testosterone ratios were higher at 5.0 mM, suggesting that VPA inhibits the activity of P450c17 (CYP17) and 17β-hydroxysteroid dehydrogenase (17β-HSD). The testosterone/estradiol ratio was higher at 0.2 mM and above, suggesting that VPA inhibits the activity of P450 aromatase (CYP19). Based on these results, we conclude that VPA shows the ovarian toxicity as suppression of follicular development with death of GCs andendocrine disorders induced by inhibition of CYP17, 17β-HSD and CYP19 activities.

The androgen signaling pathway plays a critical role in sexual differentiation during development in mammals and is one of the better understood pathways in human development. Thus it was chosen as a model pathway to evaluate the potential of HTP in vitro assays as risk assessment tools. This study examined the interaction of chemicals with the androgen receptor (AR) using in vitro cell-based transcriptional activation assays. The chemicals identified as positive agonists or antagonists were then tested in competitive binding assays to confirm receptor interaction. An initial set of about sixty well-characterized compounds with varying affinities for the AR were tested. The in vitro results from these known compounds were compared to available data from in vivo Hiroshberger assays to evaluate the predictive capacity of the in vitro assays when compared to in vivo results. About fifty unknown chemicals were also tested in vitro and evaluated using the criteria developed. Results for chemicals with known activity in vitro and in vivo indicate that most with ED 50 s lower than 10^6 M were drugs or natural steroids. The pesticides and toxic substances known to have in vivo effects via the AR fell in the 10^6 to 10^9 M range. In the 10^9 to 10^10 M ED 50 range, there was no correlation between in vitro activity and the in vivo potency. An examination of the chemicals in this range indicates that the limitations of the in vitro assays (failure to account for metabolic inactivation, activation, and half-life of a compound.) result in a high rate of “false positives” precluding their use for accurate prediction of in vivo effects. However, since there were no “false negatives” (in vitro versus in vivo) these in vitro receptor assays can be used to prioritize chemicals for additional in vitro or short-term in vivo screening for compounds that act via the AR signaling pathway in an HTP mode. Disclaimer: This abstract does not necessarily reflect EPA policy.

The cytosolic monkey (Macaca fascicularis) is a useful animal model for non-clinical developmental and reproductive toxicity studies of biopharmaceuticals. Since “enhanced” pre- and post-natal development study with detailed on in utero embryo-fetal examinations (ePPND) is becoming an increasingly common study design, post-natal linear growth data is essential to adequately evaluate potential risk to the infant. The goal of this presentation is to categorize similarities and differences in natural delivery data and infant development parameters during early stage of post-birth between cytosolic monkey monkeys of different origins. Mean gestation length in Chinese (CH), Cambodian (CA) and Indonesian (ID) monkeys were 162 ± 9 (mean ± SD), 160 ± 11 and 159 ± 7 days, respectively, and were comparable amongst three origins. Incidence of stillbirth in CH (9.3%) was lower than in CA (21.4%) or ID (16.0%). Infant mortality (death or euthanized for humane reasons within one month post-birth) was low in CH (2.6%) and CA (0.0%), but higher in ID (14.3%). Average maternal body weight at term in ID was approximately 22% lower than CH, while CA was comparable with the CH. Infant body weights at birth and one month after birth in ID was also approximately 10-11% lower than CH. Infant external linear measurement parameters (head width, crown-rump length, and head and chest circumferences) as well as peripheral blood lymphocyte phenotype data (CD3+, CD3+CD4+, CD3+CD8+, CD3+CD16+ and CD3+CD20+) were comparable within all three origins. In conclusion, there are some differences in stillbirth ratio, infant mortality, and maternal and infant body weights amongst the origin of animals, but no clear differences were noted in infant external linear measurement and peripheral blood lymphocyte phenotype data. These facts should be taken into consideration when designing or conducting a PPNP study.
dose of 1/20 LD50 (400 mg/kg, b.wt) for 65 days via gastric intubation. DBP induced a significant decrease of sperm motility percentage, sperm cell count and a significant increase in sperm abnormalities including detached head, bent tail, looped sperm and stunted sperm. Also there is a significant decrease in the level of testosterone hormone accompanied with increase of LH hormone in treated group. So, DBP adversely affect the female reproduction and cause teratogenic effect. It also affect male fertility and epididymal spermatozoid characteristics.

Oxidative stress has been associated with male infertility. The transcription factor Nuclear Factor-Erythroid 2-Related Factor 2 (NRF2) regulates basal and inducible transcription of genes encoding enzymes important for protection against oxidative stress. We hypothesized that deletion of Nrf2 causes testicular and epididymal oxidative stress, which disrupts spermatogenesis. Our results show that fertility of male Nrf2-/- mice decreased significantly between 2 and 7 months of age compared to wild type and heterozygous littersmates, due to accumulating seminiferous tubule damage with increasing age. Testicular sperm head counts, epididymal sperm counts, and epididymal sperm motility in 2 month old Nrf2-/- males did not differ from wild type littermates; however, by age 6 months, Nrf2-/- males had 44% lower testicular sperm head counts, 65% lower epididymal sperm counts, and 66% lower epididymal sperm motility than wild type males. Testicular histology showed that the most severely affected 7 month old Nrf2-/- males had many vacuolated semiferous tubules with few germ cells; Sertoli cells were spared. Compared to wild type littersmates, 3 to 4 month old Nrf2-/- males had significantly elevated levels of testicular and epididymal lipid peroxidation and testicular germ cell apoptosis, and decreased enzymatic activity and/or mRNA levels of several glutathione-S-transferases, of the subunits of glutamate cysteine ligase, the rate limiting enzyme in glutathione synthesis, of glutathione peroxidase, and of glutathione reductase. These results provide evidence that oxidative stress has deleterious effects on the testis and epididymis and demonstrate a critical role for the transcription factor NRF2 in preventing oxidative disruption of spermatogenesis. Funded by UC Irvine CORCLR R01ES012893.

In utero and lactational (IUL) exposure to 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD)—an endocrine disruptor and pollutant—has a significant long term negative effect on female reproduction in rodents. TCDD exerts this disruptive effect, at least in part, at the level of the ovary to reduce the growth of antral ovarian follicles; however the mechanism is not clear. Herein we questioned whether IUL exposure to TCDD reduced steroid biosynthesis by this cohort of antral follicles by disrupting the activity of aromatase. Immunohistochemistry was used to examine whether small antral ovarian follicles express the Aromatase Receptor (AHR)—also known as the TCDD receptor. The effect of IUL exposure to TCDD on: 1) aromatase protein levels and activity in granulosa cells from ovarian antral follicles, 2) steroid biosynthesis by explanted antral ovarian follicles and 3) AHR protein levels in ovarian tissue was also examined. The AHR was present in ovarian follicles at all stages of growth supporting the postulate that small antral ovarian follicles are sensitive to TCDD-AHR induced toxicity. Surprisingly, we found that steroid synthesis by cohorts of antral follicles explanted from TCDD exposed animals was not compromised nor was aromatase activity. However, we did find that early exposure to TCDD resulted in reduced levels of the AHR in ovarian tissue. This finding is significant because in addition to mediating the TCDD toxic response, antral ovarian follicles also require the AHR for normal growth—mediated by a yet to be identified natural AHR ligand. Thus, we suggest that TCDD by reducing levels of the AHR may cause a block in the growth of a subset of small antral ovarian follicles, contributing to a well characterized negative TCDD effect on female reproduction in rodents.
**386** IMPAIRMENT OF FETAL MOUSE PROSTATE DEVELOPMENT BY 2, 3, 7, 8 TETRACHLOROBENZENO-P-DIOXIN (TCDD) IS LINKED TO A DEFECT IN β-CATENIN SIGNALING.

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Development of C57BL/6j mouse prostate is perturbed by in utero exposure to 2,3,7,8-tetrachlorobenzene-p-dioxin (TCDD). TCDD inhibits prostatic ductal bud formation in ventral and dorsolateral regions of the urogenital sinus (UGS) to cause ventral prostate agenesis and reduce dorsolateral prostate size. This study tested the hypothesis that TCDD impairs WNT/β-catenin signaling to inhibit ventral prostatic bud formation. In wild type control male fetuses, β-catenin-responsive gene expression was observed during prostatic bud formation in UGS basal epithelial cells. TCDD exposure (5 μg/kg, maternal dose) on embryonic day (E) 15.5 reduced the frequency of UGS basal epithelial cells with detectable levels of β-catenin regulated proteins on E16.5. Targeted β-catenin deletion in UGS basal epithelial cells reduced β-catenin responsive gene expression and impaired prostatic bud formation in control male fetuses, while targeted β-catenin stabilization in these cells protected male fetuses from TCDD-induced ventral prostatic bud inhibition. We are beginning to explore the mechanism by which TCDD impaired β-catenin signaling. We reported previously that the non-canonical WNT5A phenocopied the actions of TCDD in cultured UGS tissues by impairing ventral prostatic bud development. We also showed that inhibition of WNT5A signaling restored ventral prostatic development in UGS tissues cultured in media containing TCDD. We now show WNT5A and TCDD both decrease β-catenin-responsive protein abundance in cultured UGS epithelium. Thus, impaired WNT and β-catenin signaling may be the mechanism by which TCDD disrupts prostatic bud formation in C57BL/6j mice (Supported by NIH grants ES01332 and DK083425)

**387** EFFECT OF ANTI-MÜLLERIAN HORMONE ON 4-VINYLCYCLOHEXENE DIEPOXIDE-INDUCED OVOSTICYTIC IN URTED PND 4 RAT OVARIIES.

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Anti-Müllerian hormone (AMH), also known as Mullerian inhibiting substance, is known for its role in mediating the toxic effects of numerous environmental contaminants. Recent data also indicate that the AHR plays a role in normal ovarian physiology. Specifically, recent studies from our lab indicate that primordial follicle formation may be occurring faster in AhR null mice (AhRKO) and that the AHR may be regulating genes essential for germ cell survival and follicle assembly. Problems in follicle assembly and maintenance during neonatal life can lead to infertility. However, the role of the AHR in neonatal ovarian development is relatively unknown. Thus, this study was designed to test the hypothesis that on postnatal day 8 (PND8), when most primordial follicles have fully formed in the mouse and the early stages of follicle growth have commenced, there are changes in expression of genes involved in regulating proliferation and apoptosis between AhRKO and wild type (WT) mouse ovaries. To test this hypothesis, ovaries were collected from AhRKO and WT mice on PND8 and subjected to Affymetrix GeneChip® Mouse Genome arrays to compare the expression of more than 20,000 genes. A comparative analysis revealed differential expression of 602 genes: 387 genes were up-regulated, whereas 215 genes were down-regulated in the AhRKO compared to WT ovaries. Nearly 8% of the differentially regulated genes play roles in proliferation and cell death. Interestingly, a gene responsible for negative regulation of cell death in granulosa cells, BCL2-like 1 (Bcl2L1), was up-regulated in the AhRKO ovaries compared to WT ovaries. Another gene known to play a role in maintaining the size and longevity of the primordial follicle pool, chemokine (C-X-C motif) receptor 4 (Ccr4), was up-regulated in AhRKO ovaries compared to WT ovaries. Collectively, these data suggest that the AHR regulates genes that are important in the survival and maintenance of the primordial follicle pool in the neonatal ovary.

**388** THE ARYL HYDROCARBON RECEPTOR MAY REGULATE GENES INVOLVED IN PROLIFERATION AND CELL DEATH IN THE NEONATAL MOUSE OVARY.

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The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor known for its role in mediating the toxic effects of numerous environmental contaminants. Recent data also indicate that the AHR plays a role in normal ovarian

**389** THE REAL-TIME CELL ELECTRONIC SENSOR AND ITS APPLICATIONS IN ENVIRONMENTAL TOXICOLOGY.

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The Real-time Cell Electronic Sensor (RT-CES) system is a technology based on the measurement of cell-electrode impedance. Circle-on-line microelectrode arrays are coated on a glass surface and integrated into the wells of microtiter plates. Adherent cells grow on the sensors and their physiological activities affect the electronic and ionic passage. Thus, the dynamic curve recording the electrode impedance, which is displayed as cell index (CI) values, can be used to monitor cell viability, number, morphology, and adhesion. It is a new generation analytical tool that provides real-time, label-free and non-invasive measurements in cell-based assays. Our laboratory has employed the RT-CES technology in developing novel assays in environmental endocrine disrupting compound (EDC) research. Traditionally, estrogenic compounds are analyzed by the E-screen using MCF-7 (breast cancer) cells. However, it is time and labor consuming because of the long culture period (up to 10 days) and tedious cell counting process. Using the RT-CES technology, we implemented a novel parameter known as “doubling time” based on the multi-dimensional data to replace the traditional endpoint cell counts. We have successfully modified/adapted the E-screen assay to the real-time platform, thus decreasing the assay time to less than 7 days. Over 70 compounds were screened for estrogenic or anti-estrogenic properties using this method. Their ability to promote or inhibit MCF-7 growth was recorded at various levels. The results correlate well with limited published results from other researchers. Using our method and doubling time parameter, we were able to improve upon the E-Screen assay sensitivities by 2 to 20 folds depending on the individual substance. In conclusion, we have developed a novel, sensitive, reliable and high-throughput method for investigating estrogenic EDCs.

**390** RELATIONSHIP BETWEEN URINARY PHYTOESTROGENS AND IDIOPATHIC MALE INFERTILITY.

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Aim: To investigate the relationships between urinary phytoestrogens (PEs) levels, idiopathic male infertility and semen quality of Chinese adult males. Methods: Through eligibility screening procedures, 608 idiopathic infertile men and 469 fertile controls were recruited. Based on their semen volume, sperm concentration, sperm number per ejaculate and sperm motility, subjects with idiopathic infertility were further divided into “normal” and “abnormal” semen quality groups. By using UPLC-MS/MS, individual exposures to PEs were evaluated by urinary concentrations of eight PEs or their metabolites (secoisolariciresinol, SEC, enterolactone, ENL; enterodiol, END; daidzein, DA; equol, EQU; genistein, GEN; naringin, NAR; coumestrol, COU), which were adjusted by urinary creatinine (CR). Results: Most of the adult males in our study demonstrated high exposure levels of PEs. The
median CR-adjusted isoflavone concentrations were higher than those in the U.S reports, but the lignans concentrations were lower than those in the U.S reports. The CR-adjusted urinary concentrations of SEC, GEN and DAI of infertile group were significantly higher than those of control group. With the increasing SEC, GEN and DAI levels (assessed as quintiles), the odds ratios (ORs) of these semen parameters showed the similar trends. From the first quintile to the fourth quintile, there were no significant variations about the ORs, whereas from the fourth quintile to fifth quintile, the ORs increased significantly. Subjects with higher urinary concentrations of SEC, GEN and DAI were more likely involved in idiopathic male infertility with abnormal semen quality (P < 0.01, 0.05 and 0.05, respectively). Conclusion: High level urinary concentrations of SEC, GEN and DAI were found associated with increased risk of male idiopathic infertility. Furthermore, the idiopathic infertile subjects with abnormal semen might be in higher risk. These findings should be of concern because of the ubiquitous exposure of PEs.

We summarized the findings of recent animal studies on the reproductive and developmental toxicity of the degradation products of 1,1,1,2-tetrafluoroethane (HFC-134a) used worldwide and 2,3,3,3-tetrafluoropropene (HFO-1234yf) developed for new generation. Trifluoroacetic acid (TFA), carbon dioxide (CO2), carbon monoxide (CO), carbonyl fluoride (CF), hydrogen fluoride (HF) and formic acid (FA) were evaluated. Excessive CO2 is testicular and reproductive toxic, embroyotoxic and developmentally neurotoxic and teratogenic in experimental animals. Maternal exposure to CO causes prenatal and postnatal lethality and growth retardation, skeletal variations, cardiomegaly, blood biochemical, immunological and postnatal behavioral changes, and neurological impairment in offspring of several species. In very early studies, CO was reported to be teratogenic in rats and guinea pigs. The results of toxicological studies on sodium fluoride (NaF) were used to obtain a trend insight into the toxicity of CF and HF, because CF is rapidly hydrolyzed in contact with water yielding CO2 and HF. NaF is similar in kinetics and dynamics to HF. Increased fetal skeletal variation, but not malformation, was noted after the maternal administration of NaF. Rat multiple-generation studies revealed that NaF caused retarded ossification and degenerative changes in the lung and kidney of offspring. There is a lack of information about the toxicity of TFA and FA. Animal studies remain necessary for the risk assessment of chemicals because it is difficult to find alternative methods to determine the toxic effect of chemicals.

Although an age-effect on reproduction outcome is well documented, information on relevant parameters for a successful mating programme in macaque species is scarce. Thus, we conducted a cohort study on cumulative pregnancy rates in the cynomolgus monkey, currently the predominant nonhuman primate model for preclinical safety assessment. A retrospective analysis was performed on 3870 matings with 1489 females and 82 males. Ovarian cycles were monitored and animals were cohabitated during the anticipated ovulation event. Pregnancy rates were calculated by comparing different cohorts (n=5) of females over the last 4 years grouped for age and body weight at mating start. Single (first time)-mated females were compared with multi-mated females to assess bias of several mating attempts with 1489 females and 82 males. Ovarian cycles were monitored and animals were caged during the anticipated ovulation event. Pregnancy rates were calculated by comparing different cohorts (n=5) of females over the last 4 years grouped for age and body weight at mating start. Single (first time)-mated females were compared with multi-mated females to assess bias of several mating attempts per female. Analysis of male mating success was also included. Data were explored according to age, season, and weight of both sexes. Overall, 376 pregnancies (25.7%) were achieved in single-mated females. For multi-mated females, 946 pregnancies (35.6%) were achieved per animal. Thirty-eight (38%) per ovulatory cycle were attained during the first 2-3.5 (mean ± SD) matings. Subgroup analysis by age revealed a cumulative pregnancy rate of 0% before 3.0 years, 18.2% until 3.5 years, 20.9% until 4.0 years, 25.3% until 4.5 yrs and 30.4% until 5.0 years and older. Pregnancy age distribution was independent of number of matings and the age distribution at first mating was comparable. Female body weight at mating was of minor relevance for pregnancy success except for females exceeding 5.2 kg with a 50% reduced pregnancy rate. The males used in the study had an age range of 5.2 to 14.5 years with a median of 7.6 years and a weight range of 5.3 to 14.1 kg with a median of 7.6 kg. Male factors contributing to the pregnancy outcome - besides full spermatogenesis and ejaculated sperm - could not be identified. In summary, unlike age, neither female body weight, different male partners, nor season had a significant effect on pregnancy rates. This study demonstrates the impact of female age on colony breeding outcome. Healthy female animals older than 3 years are mandatory for a successful mating programme.
causes ovarian toxicity is not known. Since MXC binds to estrogen receptors (ESR), we hypothesized that MXC may be causing slow follicular growth and atre- sia of antral follicles via the estrogen receptor alpha (ESR1) pathway. To test this hypothesis, we generated and validated a transgenic mouse model in which ESR1 is overexpressed in several tissues (ESR1−OE), including the ovaries. To determine whether ESR1 overexpression affects the ability of mice to respond to MXC, antral follicles from controls and ESR1−OE mice were cultured with either the vehicle dimethylsulfoxide (DMSO) or MXC (1−100 μg/mL). A no-treatment group was also included for control purposes. The growth of the follicles was monitored every 24 h for a period of 96 h. The data show that the follicles from controls and ESR1−OE mice treated with 10 and 100 μg/mL MXC are more atretic than DMSO−treated follicles. Further, in both controls and ESR1−OE mice, follicles treated with 1 μg/mL MXC (MXC1) had less growth compared to the DMSO−treated follicles. Interestingly, the follicles from ESR1−OE mice that were treated with MXC1 had significantly less growth compared to the follicles from control mice that were treated with MXC1 (Controls: DMSO = 129.56 μm ± 3; MXC1 = 127.35 μm ± 4.3; ESR1−OE: DMSO = 150.76 μm ± 2.8; MXC1 = 112.44 μm ± 1.8; n = 5; p < 0.05). These data suggest that ESR1 overexpression may be increasing the sensitivity of antral follicles to MXC−induced slow growth and atresia. Support: NIH R21ES13061, RO1ES12893 and an Eli Lilly Fellowship in Toxicology.

**396 DEVELOPMENTAL TOXICITY EVALUATION OF 3, 3′, 4, 4′-TETRACHLOROAZOBENZENE (TCAB) IN SPRAUGE-DAWLEY RATS.**


TCAB is formed as a by−product in the manufacture of 3,4−dichloroaniline and its herbalicidal derivatives Propanil®, Linuron®, and Dintrouron®. In a previous reproduction study, TCAB was found to reduce F1 and F2 litter size and pup weight. Cross breeding of treated animals with controls showed effects were dam−mediated. A second study was conducted using the same species/strain and doses to more specifically assess the developmental toxicity of TCAB. Adult female Sprague−Dawley rats (25 timed−mated/group) were administered 0, 1, 3 or 10 mg/kg/day by gavage from 2 weeks before cohabitation with untreated males until the day of gestation. F1 pups were weaned at PND 21 and paired with untreated males for the production of F2 pups. F1 and F2 pups were weaned at PND 21 and PND 28, respectively. F1 females were cohabitated with untreated males and F2 females were bred to untreated males. Dams were killed at the end of lactation, and uterine contents were evaluated at necropsy. Following C−section, fetuses were removed, counted, and weighed. The results showed that TCAB caused postimplantation loss and stillborns, and total litter losses in two P2 dams. The maternal and developmental effects were evaluated at the NOEL for systemic toxicity was 0.01%.

**398 THE ABILITY OF THE ARYL HYDROCARBON RECEPTOR TO REGULATE OVARIAN FOLLICLE GROWTH IS INFLUENCED BY FOLLICLE−STIMULATING HORMONE.**

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The aryl hydrocarbon receptor (AHR) is a ligand−activated transcription factor that mediates the toxicity of dioxins. The AHR has also been shown to regulate physiological functions, including ovarian function. Previous studies have shown that Ahr−deletion (AHRKO) results in slow growth of antral follicles, which are the functional units of the ovary. Further, we previously showed that growth of follicles is decreased in AHRKO antral follicles compared to wild−type (WT) antral follicles between post−natal day (PND) 30 and 53, times when mice undergo puberty and start cycling. We also showed that growth of follicles in AHRKO ovaries recovers to WT levels after PND 53, but the reasons are unknown. Since follicle−stimulating hormone (FSH) promotes follicle growth after the onset of puberty, we hypothesized that differences in growth according to stage of sexual maturity in AHRKO follicles is due to the shift of a lack of FSH stimulation of follicles before puberty to a constant FSH stimulation of follicles after puberty. This study evaluated whether this is the case. Antral follicles from WT and AHRKO ovaries on PND 30, a time when follicle growth is impaired in AHRKO follicles, were mechanically isolated and cultured in vitro with or without recombinant FSH (rFSH: 0−15 IU) for 7 days. Follicle growth was measured every 24 h and presented as percent change compared to that of day 1. AHRKO follicles treated with rFSH 0 and 5 IU had decreased growth compared to WT follicles by the end of culture (rFSH 0 IU: WT=142 ± 8.9, AHRKO=137 ± 12.7, AHRKO−OE=166 ± 17.3; n=3 cultures; p<0.05). Interestingly, AHRKO follicles treated with rFSH 10 and 15 IU grew in a similar manner to WT follicles by the end of culture (rFSH 10 IU: WT=145 ± 9.4, AHRKO=154 ± 12.3 and rFSH 15 IU: WT=142 ± 12.7, AHRKO−OE=166 ± 17.3; n=3 cultures; p>0.05). Collectively, these data suggest that differences in growth of Ahr−deleted follicles according to stage of sexual maturity may be mediated by FSH. Support: NIH HD040725.

**399 METHOXYCHLOR MAY CAUSE TOXICITY THROUGH THE ARY HYDROCARBON RECEPTOR PATHWAY.**

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Methoxychlor (MXC) is an organochlorine pesticide used against pests and insects that attack crops, gardens, vegetables, pets, and livestock. MXC has been shown to reduce fertility, and to cause persistent estrus and ovarian atrophy. Further, MXC inhibits growth and induces atresia of mouse antral follicles in vitro. Little is known, however, about the mechanisms by which MXC causes slow growth and atresia of antral follicles. Several studies indicate that some pesticides act through the aryl hydrocarbon receptor (AhR) pathway and one study has shown that MXC binds to the AhR in liver cells. Previously, we showed that MXC inhibits growth of antral follicles through the AhR pathway, but we did not determine whether MXC causes atresia through the AhR pathway. Thus, the current study tested the hypothesis that MXC causes atresia of antral follicles through the AhR pathway. Antral follicles were mechanically isolated from ovaries of C57BL/6 female wild−type (WT) and AhR null (AHRKO) mice aged 30−35 days. The isolated antral follicles (10−15 per treatment) were cultured in dimethylsulfoxide (control; DMSO) or MXC (1, 10, 100 μg/mL) for 168 h at 37°C and 5% CO2 in α−minimum essential media. After culture, the follicles were fixed in Dietrich's solution, embedded in plastic blocks, sectioned in 2 μm sections, stained with Lee's methylene blue−basic fuchsin, and graded for atresia on a scale of 1 to 4 based on the percentage of apoptotic bodies (1−0%, 2−<10%, 3−10−30%, and 4−>30% apoptotic bodies). The results indicate that MXC (10,100 μg/mL) significantly induced atresia in WT antral follicles (DMSO=1.83 ± 0.38, MXC10 μg/mL=3.29 ± 0.36, MXC1000 μg/mL=4±0.4, p<0.05, n=4). Conversely, MXC (10 μg/mL) did not significantly induce atresia in AHRKO follicles (DMSO=1.2±0.14, MXC10 μg/mL=2.11±0.29, n=4). These...
data indicate that AhR deletion protects antral follicles against MXC induced atresia. Collectively, these data suggest that MXC may cause atresia through the AhR pathway. Support: NIH R01 ES012893, NIH R01 HD047275, and the Environmental Toxicology Program at UIUC.

**400 DECREASED REPRODUCTIVE INDICES IN H-NAG-1 (GDF-15) MICE CORRELATED TO ALTERATIONS IN SERUM LEPTIN LEVELS.**

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The nonsteroidal anti-inflammatory drug (NSAIDS)-activated gene (NAG-1) is induced by a variety of chemopreventive and anti-inflammatory agents. Heterozygous male and female transgenic mice with multiple copies of the human-NAG-1 gene were mated to wild type (WT) C57BL/6 mice. Female heterozygous h-NAG-1 mice have decreased rates of pregnancy (49%), weaning (5.0 weaning/litter), and decreased number of live pups per mating (0.25) as compared to WT C57BL/6 female mice mated to heterozygous NAG-1 male mice (58%). Although heterozygous h-NAG-1 female mice consume similar amounts of feed/kg body weight (1.5 +/- 0.4) as WT mice (1.4 +/- 0.21), NAG-1 mice weigh 20% less and have less abdominal fat than WT mice of the same age. This data suggests differences in energy metabolism. Adipokines leptin and adiponectin play critical roles in energy homeostasis. Because ob/ob mice have mutations in the leptin gene, altered energy metabolism, and are infertile, serum m-leptin levels were quantified by ELISA. m-Leptin levels in heterozygous female NAG-1 mice were significantly decreased relative to WT female mice. Serum h-NAG-1 levels were quantified by ELISA; no h-NAG-1 was found in WT mice, while mean levels in transgenic female mice were 12.0-13.0 ng/ml. These data suggest that decreased reproductive fertility and fecundity in female h-NAG-1 mice may be due to alterations in suppression of leptin pathway. Funding by NIEHS Contract No. N01-ES-35513.

**401 TREATMENT OF LACTATING DAMS WITH HMPCC CAUSES REVERSIBLE SKIN PEELING IN RAT OFFSPRING.**

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HMPCC (4-(4-hydroxy-4-methylpentyl)-3-cyclohexen-1-carboxaldehyde) is a widely used fragrance material. In a one-generation reproduction study, male and female rats were dosed via gavage with 0, 25, 100, or 500 mg/kg/day HMPCC according to OECD Testing Guideline 415. At 100 and 500 mg/kg/day, skin peeling was observed in the offspring. Two follow-up studies were subsequently conducted to investigate this effect. In the first study, rats were dosed via gavage with 0 or 500 mg/kg/day HMPCC only during gestation or lactation. All pups were evaluated through lactation day 21 (DL 21). From dams treated during gestation, pups had flaking skin (minor) which was transient and all pups recovering by DL 16. From dams treated during lactation, pups had peeling skin, the more severe observation, which persisted to necropsy. In the second study, lactating rats were dosed via gavage with 0, 10, 25, or 500 mg/kg/day HMPCC during lactation. Milk samples were analyzed for HMPCC on DLs 14 and 21. Pups were weaned on DL 21 and evaluated through a postweaning recovery period of six weeks. Skin peeling was only observed in pups from dams treated with 500 mg/kg/day HMPCC. In conclusion, skin changes were observed in pups following postnatal exposure of dams to HMPCC. Skin peeling was found to be correlated with HMPCC in maternal milk and reversible within two weeks following weaning.

**402 GSTPI AND PHOSPHO-C-JUN INTERACTIONS IN 4-VINYLCYCLOHEXENE DIEPOXIDE-INDUCED OXOTOXICITY IN THE RAT OVARY.**

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The occupational chemical 4-vinylcyclohexene diepoxide (VCD) selectively destroys ovarian small pre-antral follicles in rats and mice. Exposure of postnatal day 4 (PND4) F344 rat ovaries in vitro to VCD (d6) results in primordial and small primary follicle loss (P < 0.05) via apoptosis. VCD is detoxified through glutathione conjugation, catalyzed by glutathione S-transferase (GST) proteins. In other systems, GSTs also regulate signal transduction pathways. For example, the GSTp1 isoform and pro-apoptotic c-Jun form protein complexes which disassociate upon xenobiotic exposure allowing GSTp1 to catalyze Phase II metabolism and c-Jun to induce apoptosis. VCD (30 μM) exposure increased (P < 0.05) GSTp1 expression (mRNA and protein) in cultured PND4 rat ovaries by d8. Further, in vivo dosing of rats with VCD (80mg/Kg/d; 15d, i.p) increased (P < 0.05) phosphorylated c-Jun (P-c-Jun) in ovarian small pre-antral follicles. Thus, the present study investigated temporal effects of VCD on ovarian GSTp1-c-Jun protein complexes. PND4 F344 rat ovaries were incubated in control medium or medium containing VCD (30 μM) for 4-8d (n=3; 20 ovaries per pool). Co-immunoprecipitation with anti-p-c-Jun antibody was followed by Western blotting with anti-GSTp1 antibody. Ovarian GSTp1-c-Jun protein complexes were observed. Relative to control, GSTp1 bound to p-c-Jun was unchanged by VCD exposure after 4d, increased by 16% (P < 0.05) on d6, and returned to control levels by d8. Conversely, unbound GST p1 was increased (P < 0.05) by VCD exposure on d4 (6%) and d8 (47%), but unchanged on d6. Thus, modifications to GSTp1-c-Jun protein complexes occur at the time of VCD-induced follicle death supporting a detoxification role for GSTp1. Further, these findings demonstrate that ovarian GST proteins may play dual protective functions by also regulating pro-apoptotic proteins. (Supported by K99ES016818; ES09246 and Center Grant ES06694).

**403 EFFECT OF MATERNAL EXPOSURE TO THIAMAZOLE ON BEHAVIORAL DEVELOPMENT, LEARNING, AND MEMORY IN INFANT CYNOOMOLGUS MONKEY.**

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Thiamazole is a widely used antithyroid agent; however, it has been reported to inhibit fetal thyroid function and postnatal hypothryroidism in neonates. Thiamazole was administered orally to cynomolgus monkeys at doses of 0, 2 and 3.5 mg/kg/day during the late gestational period (from GD 120 to 150) to investigate effects on behavioral development, and on learning and memory in infant. As a behavioral development test battery, interactive behavior between the mother and the infant was monitored, and eye contact with an observer by the infant, a quantifiable and reliable behavioral measure, was examined. To examine cautiousness, fear, and interest in humans, Learning and memory were examined by a finger maze, which has 2 phases (learning and memory). The reward could be moved with the fingers along the maze to reach a goal, from which it can be retrieved by the animal. In the thia- mazole-treated groups, contact behavior with the mother (e.g. ventral contact, clinging, nipple contact) similar to that in the control group was observed on postnatal day (PND) 25. On PND 170, foregrowing behavior did not decrease, and independent and exploratory activities (e.g. locomotion, environmental exploration) were almost not observed. Moreover, eye contact was less frequent than that in the control group, and the response to humans was very poor. In the learning phase of the finger maze test, there were no significant differences between the thiamazole-treated and the control group. Conversely, scores for the memory phase in the thiamazole-treated group were significantly lower than that in the control group. In conclusion, these results suggest the possibility that maternal exposure could affect behavioral development, and learning and memory, and these evaluating methods are considered to be useful for in cynomolgus monkey infants.

**404 IN VITRO EMBRYOTOXIC POTENTIAL OF ALBENDAZOLE AND ALBENDAZOLE SULFOXID ON RAT EMBRYOS IN CULTURE.**

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Albendazole is a potent anthelmintic used in human and veterinary medicine. The substance acts as an inhibitor of tubulin polymerization and causes developmental abnormalities during embryogenesis. Albendazole has been reported to be terato genic in rats; however, it is unclear whether this effect is caused by the parent compound or a metabolite.

We studied the effects of albendazole and its sulfoxide metabolite in a rat whole embryo culture system to characterize their embryotoxic potential. Both substances were tested alone and in combination at a ratio of 50:50 to mimic the metabolic situation in vivo. We cultured rat embryos from gestational day 9.5 for 48 hours and exposed them to albendazole, albendazole sulfoxide or the mixture at concentrations between 0.01 and 5.0 μM. Four embryos were cultivated in one bottle containing 6 ml heat-inactivated and sterile-filtered serum mixture for WEC (Biochrom AG), composed of 85% serum (90% bovine serum and 10% rat serum),
EMBRYOTOXIC POTENTIAL OF MYCOPHENOLIC ACID IN TWO IN VITRO TESTS.

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Mycophenolate mofetil is a widely used immunosuppressive drug which recently has been categorized as a human teratogen. So far it has not been studied in embryotoxicity in vitro assays. Therefore, we evaluated the embryotoxic potential of mycophenolic acid in two validated in vitro assays: the rat whole embryo culture and the murine embryonic stem cell test. Rat embryos cultured from gestational day 9.5 for 48 hours with the drug showed dysmorphogenic development at concentrations of 250 μg mycophenolic acid/l medium and higher. At concentrations of 750 μg/l and more all rat embryos exhibited malformations. The main effects were defective yolk sac blood circulation, neural tube defects, malformations of the head and heart anomalies. The exposed embryos showed a concentration-dependent decrease in protein content, crown-rump length, number of somites and morphological score. The murine embryonic stem cell test was slightly more sensitive. Proliferation and differentiation of the ES-D3-cells were significantly impaired at concentrations of 31 and 125 μg mycophenolic acid / l medium, respectively. In the differentiation assay, at a concentration of 125 μg mycophenolic acid / l medium and more, the number of wells with differentiated cardiomyocytes significantly decreased. Additionally, a cytotoxicity assay with bulk/cell 3T3 mouse fibroblasts was used to compare the proliferation and vitality of embryonic cells with adult cells. In the bulk/cell 3T3 cytotoxicity assay the number of vital mouse fibroblasts significantly decreased at a mycophenolic acid concentration of 62 μg/l and more. In conclusion, by using two validated in vitro tests we showed that mycophenolic acid exhibits a pronounced embryotoxic potential at cytotoxic concentrations. This result supports the use of these tests to detect human teratogens.

KISSEPTIN: A NEW PATHWAY TO IDENTIFY AND CLASSIFY ENDOCRINE DISRUPTING COMPOUNDS?


There is mounting evidence that the incidence of precocious puberty, unexplained infertility and other adverse female reproductive health effects is increasing. It has been proposed that this is due, at least in part, to exposure to endocrine disrupting compounds (EDCs). The specific mechanisms by which EDCs impact female reproductive health are not yet fully understood and consensus on an adequate method for predicting EDC effects in humans has not been reached. Here we show that disruption of hypothalamic kisspeptin signaling pathways is associated with advanced puberty and premature aromatization, making this system a potentially good biomarker for human endocrine disruption. Kisspeptins (KISS) are a newly discovered family of peptides that, through the kisspeptin receptor, act directly on gonadotropin releasing hormone (GnRH) neurons to regulate GnRH secretion. Initially discovered in humans, similar systems have now been well characterized in other mammalian species. In the mouse brain, two major populations of KISS neurons: one surrounding the anterior periventricular nucleus (AVPV) and one in the arcuate (ARC). KISS neurons in the AVPV are colocalized with both ERα and ERβ, while the ARC KISS neurons express only ERα. Only the AVPV population is sexually dimorphic with females having more kisspeptin neurons than males. Here we show that neonatal administration of estradiol benzoate (EB) to female rats reduces KISS immunoreactivity (-ir) in both the AVPV and ARC. We further show that selective agonism of ERα, but not ERβ, can best replicate the feminizing impact of estrogen. Finally, we assessed the impact of neonatal exposure to genistein (a phytoestrogen), or bisphenol-A (BPA, a plastics component) on KISS in the AVPV and ARC and found that each could produce region specific feminizing effects. We propose that examination of KISS signaling pathways could provide valuable information about a compound’s potential endocrine disrupting properties as well as insight into whether the mechanistic pathway is through ERα or ERβ.

MALE REPRODUCTIVE PARAMETERS IN A RAT TWO-GENERATION REPRODUCTION STUDY OF AMMONIUM PERFLUOROOCTANATE.

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Perfluorooctanoate (PFOA) is an industrial surfactant used in the manufacture of fluoropolymers. The environmental and metabolic stability of PFOA together with its presence in human blood and long elimination have led to extensive toxicological studies. Two recent publications based on observations from the Danish general population have reported a negative association between serum PFOA levels in young adult males and their sperm counts; and a positive association among women with time to pregnancy. A rat reproduction study was published (2004) in which no effects on reproduction were observed at doses up to 30 mg PFOA/kg body weight. In order to place the recent human epidemiological data in perspective, we are providing the detailed male reproductive parameters from the two-generation study, including sperm quality and testicular histopathology. Sperm parameters from the two-generation study in all PFOA treatment groups were normal and reflected the normal fertility observations in these males. No evidence of altered testicular and sperm structure and function was observed at mean group serum PFOA concentrations ranging up to approximately 50,000 ng/mL. Evidence from workers, where mean PFOA levels ranged from two to four orders of magnitude higher than concentrations found in the general population, did not show any consistent changes in reproductive hormonal levels that could be associated with a decrease in reproductive function. In addition, male monkeys given daily capsules containing PFOA for six months had no alterations in testicular structure or sex hormones at group mean serum concentrations ranging up to 110,000 ng/mL. Given that median serum PFOA in the Danish cohorts was approximately 5 ng/mL, it seems unlikely that levels observed in the general population, including those recently reported in Denmark, could be associated with a real decrease in sperm.

ESTROGEN LIKE EFFECTS OF CADMIUM IN MICE: ARE THEY MEDIATED VIA NUCLEAR ESTROGEN RECEPTOR SIGNALING?

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Cadmium (Cd) is a ubiquitous toxicant of environmental concern. At environmentally relevant doses, Cd has been shown to induce several well-characterized in vivo estrogenic responses like increased uterine weight, hyperplasia and hypertrophy of endometrial lining and induction of uterine progesterone receptor expression. Several in vitro studies suggest that Cd-induced estrogenic effects may be mediated via estrogen receptors (ERs). However, it is unclear whether observed effects are mediated via classical ER-signaling or other nuclear signaling pathways. We investigated the estrogenic effects in relation to CdCl2 exposure using ERE-luc mice. Immature mice were subcutaneously exposed to 5, 50 and 500 μg CdCl2/kg b.w., sterile PBS or EE2 for 3-consecutive days. Onset of puberty (vaginal opening), body and organ weight, histology of uterine, Cd tissue retention and luciferase expression analysis were performed. We found that EE2 significantly increased the uterine weight as well as luciferase expression in various tissues along with early onset of puberty. Cd did not significantly increase the uterine wet weight in any of the dose groups. No onset of puberty was observed. Luciferase expression data showed no clear effects either in reproductive or in non-reproductive organs after CdCl2 treatment. Interestingly, height of the uterine epithelium was significantly increased among EE2 as well as CdCl2 treated groups. Western blot analysis showed a dose dependent activation of MDM2; however ERK was highly activated only in low dose CdCl2 group. In conclusion, our data suggest that Cd can exert some estrogen-like-effects in immature female mice at the doses investigated; however the effects are not likely to be mediated via nuclear estrogen receptors.

BACKGROUND DATA FROM DEVELOPMENTAL TOXICITY STUDIES IN THE RABBIT.


The rabbit may be chosen as an animal model in developmental toxicity studies, which are particularly conducted for the development of vaccines intended for use in women of childbearing potential and adolescents (FDA Guidance for Industry, 2009).
levels were similar throughout the epididymis. Immunohistochemistry using antisera against both forms of abcb1 indicated minimal immunostaining in the epithelial cells or spermatozoa in the caput epididymidis, and progressively increased in the corpus and cauda. Intercellular junctions in the epididymis were detected in the distal caput, corpus and cauda. This was confirmed by western blot analysis. To assess if abcb1 was inducible by xenobiotics, rat epididymal cells (RCE) were exposed to different concentrations of nonylphenol (NP) or doxorubicin (DOX). RCE cells exposed to 20 μM NP and 500 ng/ml DOX revealed a significant induction of both abcb1a and abcb1b mRNA and abcb1 total protein, suggesting that ABC efflux transporters are inducible in the epididymis. The unique expression profile and induction of abcb1a and abcb1b in the epididymis suggests an important role for these proteins as a defence mechanism against xenobiotics in the epididymis. Supported by Environment Canada, CIHR and Armand-Frappier Foundation.

412 ASSESSMENT OF ORGANOTYPIC EPIVAGINAL™ TISSUE MODEL TO SCREEN IRRITATION POTENTIAL OF CHEMICALS.
C. Cannon, S. A耶淋湖icine, K. LaRosa and M. Klausner, MatTek Corp, Ashland, MA.

A predictive test system for assessing the vaginal irritation of chemicals and formulations will have far reaching application in industries involved in feminine care products. The vaginal mucosa is commonly exposed to chemicals or therapeutic agents that can cause irritation/inflammation and increase the susceptibility to infections such as HIV-1 and HSV-2. Hence, chemical/formulation or therapeutic agents that induce vaginal irritation are a concern for industrial and cosmetic industries. Traditionally, testing has been performed using the rabbit vaginal irritation (RVI) assay. In the current study, we investigated use of the organotypic, highly differentiated Epivaginal tissue as a non-animal alternative. Epivaginal tissues were exposed to N=6 chemicals at 3 concentrations for 1, 3, and 6 hrs. The effects of single or repeat application on tissue viability (MTT assay), barrier disruption (measured by trans-epithelial electrical resistance, TEER, or sodium fluorescein, FL, leakage), and inflammatory cytokine release (IL-1α, IL-1β, IL-6, and IL-8) were examined. When compared to untreated controls, two irritating test articles, benzalkonium chloride and nonoxynol-9, reduced tissue viability to <40%, reduced TEER to <60%, increased FL leakage by 11-24%, and increased IL-1α and IL-1β release by >100%. Four other non-irritating materials had minimal effects on these parameters. Assay reproducibility was confirmed by testing the chemicals using three different tissue production lots (coefficient of variation, CV <10%) and by using tissues derived from cells of three different donors (CV <1%). In conclusion, decreases in MTT and TEER and increases in FL and cytokine release appear to be useful endpoints for preclinical toxicity screening of chemicals/formulations. The assay method will be cost effective and reduce the use of laboratory animals for experimentation. In the future, the in vitro test method could also be useful to assess toxicity of medical devices, perform drug permeation studies, and serve as an in vitro alternative test for the RVI assay.

413 AKT/FOXO1 PATHWAY, BUT NOT ESTROGEN RECEPTOR ALPHA (ERα) OR BETα (ERβ) IS ASSOCIATED WITH FENVALATE (FEN)-INDUCED PROLIFERATION OF HUMAN UTERINE LEIOMYOMA (UTLM) CELLS AND UTERINE SMOOTH MUSCLE CELLS (UTSMC).
X. Gao, L. Yu, L. Castro, A. B. Moore and D. Dixon, NIEHS, Research Triangle Park, NC.

Our previous data showed that Fen promotes cell cycle progression from G1 to S phase in human UtlM cells and UTSMC resulting in cell proliferation. The purpose of this study was to explore possible molecular mechanisms associated with this effect. A luciferase reporter gene assay was used to directly measure the estrogenic action of Fen in both cell types. The competitive binding affinity of Fen to ERα and ERβ was examined using fluorescence polarization. Real-time RT-PCR, western blot analysis (WB) and/or confocal immunofluorescence microscopy were utilized to detect the expression of p27kip1, FoxO1 and Akt. In both cell types, the luciferase assay showed that Fen failed to induce estrogenic action at 0.1 μM to 100 μM at 24 h (p<0.05). The ER competitive binding assay further revealed that Fen did not bind to ERα or ERβ at 1 nM to 1 μM (p<0.05). Both of these excluded the possible role of an ER-mediated pathway in Fen-induced proliferation. P27kip1 mRNA levels were significantly diminished (p<0.05) by 10 μM Fen from 10 min to 24 h, in both cell types compared to controls. The majority of p27kip1 was detected in the nucleus, and a reduction in intensity of positive staining was found in both cell types treated with Fen for 24 h compared to controls. This trend was further confirmed by WB. Additionally, Fen modulated the mRNA expression
of FoxO1 similar to p27kip1. The phosphorylation of FoxO1 was largely abolished at 24 h to 72 h; whereas, activation of Akt was detected from 24 h to 72 h. Our results indicate that down-regulation of p27kip1 by Fen in UU-M cells and USmMC is associated with the Akt/FOXO1 pathway, but not ERα or ERβ. The current study suggests that Akt/FOXO1/p27kip1 could be a potential target pathway for Fen-induced proliferation, which appears to be important in the regulation of cell cycle progression, and possibly play a role in the pathogenesis of fibroids following environmental exposures.

414 ATRAZINE EXPOSURE DOES NOT IMPACT SEXUAL DEVELOPMENT AND DIFFERENTIATION IN ZEBRAFISH.

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Wild-type zebrafish (5D strain) were exposed to 0 (negative control), 0.1, (21.5 ppb), or 10 μM (2157 ppb) of atrazine from 17 days post fertilization (dpf) until 130 dpf. A treatment of 1 μM 17 beta-estradiol was included as a positive control. Each control and atrazine treatment included 8 tanks, each housing 10 fish. Control and treatment water was renewed every 3 days. Fish were observed daily and water quality parameters were monitored every three days. The negative control, positive control, and atrazine treated samples were analyzed for atrazine or 17 beta-estradiol to confirm treatment concentrations throughout the study. Following the 113-day treatment all fish were euthanized, weight and length were measured, and tissues were processed for histopathology. For each fish, gonadal tissue was observed in 15 sagittal sections. Mortality throughout the study was low and non-concentrator mortality. A significant decrease in the weight was observed in fish from the 1.0 and 10 μM atrazine treatments, but no effects on length were observed. There was a significantly larger number of female fish (74%) in the 17 beta-estradiol positive control treatment compared to the negative control, but there was no significant difference in the number of males and females in the atrazine treatments compared to the negative control. In a total of five sexually mature male zebrafish one or two primordial oocytes were observed within testes in the 0.1, 1 and 10 μM atrazine exposure groups. This response was not concentration dependent and may represent a developmental delay. Results indicate that estrogen effects sexual development and differentiation in zebrafish, but atrazine effects on sexing was not being provided by Syngenta Crop Protection (Greensboro, NC, USA).

415 N-ACETYL-L-CYSTEINE PREVENTS THE TOXIC EFFECTS OF HYPERPHOSPHATEMIA AND HYPOZINCEMIA ON THE TESTICULAR FUNCTION IN WKY, BUT NOT IN SHR/NDMCr-cp. A MODEL OF METABOLIC SYNDROME.

Y. Suzuki1, S. Ichihara1, A. Kato1, T. Yamaguchi1, Y. Yamada2 and G. Ichihara1. 1Life Science Research Center, Mie University, Tsu, Japan and 2Social Life Science, Y. Suzuki1, S. Ichihara1, A. Kato1, T. Yamaguchi1, Y. Yamada1 and G. Ichihara1.

Background: It is known that diabetes mellitus and metabolic syndrome affect testicular function. Given that patients with diabetic nephropathy are easily suffering from hyperphosphatemia and hypozincemia are clinically described, we assessed the higher phosphorus and lower zinc levels in plasma may affect testicular function using SHR/NDMc-r-cp, a rat model of metabolic syndrome. We also investigated the effects of an antioxidant, N-acetyl-L-cysteine (NAC), on the development of testicular dysfunction under such conditions.

Methods & Results: Male SHR/NDMc-r-cp and control (WKY) rats were divided into 3 groups and were fed control diet (P 0.3% w/w, Zn 0.2% w/w) or a high phosphorus or zinc-deficient diet (P 1.2% w/w, Zn 0.0% w/w) diet. The latter group was treated with either NAC (1.5 mg/g per day) or vehicle from 8 to 12 weeks of age (n = 6 or 8 for each group). The weights of testis and epididymis are prominently reduced by high-phosphate and zinc-deficient diet in both SHR/NDMc-r-cp and WKY. The high-phosphate and zinc-deficient diet significantly reduced caudal epididymal sperm count and sperm quality caused an increase in the histopathological changes in the testis and a decrease in the plasma testosterone concentration in two strains. The ratio of reduced to oxidized glutathione was decreased by the same diets. The treatment with NAC significantly prevented the toxic effects on the testicular function in WKY, but in SHR/NDMc-r-cp it could not improve the toxic effects. It also prevented a decrease in the ratio of reduced to oxidized glutathione in only WKY.

Conclusion: Dietary high phosphorus and deficiency of zinc induced testicular dysfunction. These changes resist against the protective effects by NAC in the metabolic syndrome, suggesting severe degree of organ damages in this model.

416 BISPHENOL-A AFFECTS UTERINE GENE EXPRESSION BUT HAS NO ADVERSE EFFECT ON UTERINE RECEPTIVITY IN MICE.

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Bisphenol-A (BPA) is an endocrine disruptor that has been widely used in polycarbonate plastics and epoxy resins. It has been demonstrated that BPA has adverse effects on uterine development and can alter the expression of progesterone and estrogen receptors in the uterus. Uterine receptivity is a transient state in which the uterus can accept an embryo to implant into the uterine wall. It is controlled by the ovarian hormones progesterone and estrogen. To investigate whether BPA has any adverse effect on uterine receptivity, timed pregnant wild type C57B6J 129 mixed background young adult females (3-4 each group) were treated with 0, 0.1, 1.0, and 10 mg/kg BPA (dissolved in sesame oil) daily via intraperitoneal injection from embryonic day 9 throughout nursing period when the pups were weaned at postnatal day 21. All the pups from 10 mg/kg BPA-treated mothers were born dead or dead within five days after birth. The pups from 0.1 and 1.0 mg/kg BPA-treated groups showed retarded postnatal growth. All the mothers and the female offspring (8-10 weeks old) from 0, 0.1, and 1.0 mg/kg BPA-treated groups were examined for uterine receptivity. They were mated with untreated wild type stud males. Implantation sites were visualized with Evans blue dye injection at 4.5 days post-coitus (implantation normally initiates - day 4.0, mating night as day 0). Both mothers and the female offspring had on-time implantation and even embryo spacing, indicating normal uterine receptivity. We further examined the gene expression in the day 4.5 uteri and found that a few genes were differentially expressed upon BPA treatment. Our data demonstrate that although BPA can alter uterine gene expression, it does not have an adverse effect on uterine receptivity in both treated mothers and their offspring under our experimental setting.

417 REPRODUCTIVE TOXICOLOGY ASSESSMENT WITH A MURINE ANTIBODY AGAINST THE IL-1 RECEPTOR.

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Interleukin-1 (IL-1, α and β) has been implicated in host response to infection and injury and is an important mediator of inflammatory diseases such as rheumatoid arthritis. IL-1 induces the production of pro-inflammatory molecules including cytokines such as IL-6 and tumor necrosis factor (TNF). Inhibitors of this pathway have promise in treating inflammatory diseases such as rheumatoid arthritis. To evaluate the potential reproductive effects of IL-1 inhibition, a murine antibody was developed for evaluation in fertility and embryo-fetal development studies. Mu108RaXMu, a chimeric antibody that binds with high affinity to the murine IL-1R1. This antibody inhibits the in vivo production of IL-6 induced by IL-1 by 50% or greater at doses of 0.1 to 30 mg/kg.

Fertility control was assessed in male and female mice (25 mice/group) that were administered Mu108RaXMu via intravenous injections of 0, 25, 100, or 300 mg/kg/dose twice per week. There were no effects on male or female fertility up to 300 mg/kg/dose. A satellite group of male mice (20/group) was dosed for 3 months and allowed a 12-week recovery period. These animals were evaluated for standard toxicology endpoints and served as an assessment of repeated dose toxicity. Significant increases in white blood cells were observed at all doses by the end of the 12 week recovery. Exposure to Mu108RaXMu was less than dose-proportional and no antibodies against Mu108RaXMu were detected. Presumed pregnant female mice (25/group) were administered Mu108RaXMu via IV injection at doses of 0, 25, 100, or 300 mg/kg on GD 6, 9, 12, and 15. Mu108RaXMu was well-tolerated, with no evidence of maternal or fetal toxicity at doses up to 300 mg/kg/day. These studies support that blocking the IL-1 type I receptor does not have adverse effects on fertility or embryo-fetal development in mice.

418 THE USE OF A COMBINED 2-GENERATION REPRODUCTION AND DEVELOPMENTAL NEUROTOXICITY TEST TO EXAMINE A PLASTICIZER FOR POSSIBLE HORMONE DISRUPTING EFFECTS.

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Some phthalates have documented negative effect on the endocrine system and there is an urgent need for alternative plasticizers. Therefore a regulatory required combined 2-generation reproduction toxicity test (OECD 416) and a developmen-
At this stage the plasticizer has shown no significant toxicity to either adults or offspring or hormone disrupting effects at dose levels up to 15000 ppm by dietary route. The histopathology of nervous system tissues and, additionally, pre-weaning motor activity and function, auditory startle habituation, learning and memory and motor activity was measured in F2 offspring. Data obtained in this study show no reproductive or hormone disrupting effects at dose levels up to 15000 ppm by dietary route. At this stage the plasticizer has shown no significant toxicity to either adults or offspring which makes it a relevant substitute for existing phthalates.

2, 4-DICHLOROPHENOXYACETIC ACID (2, 4-D): EVALUATION OF SYSTEMIC TOXICITY IN A DIETARY EXTENDED ONE-GENERATION STUDY IN CRL:CD(SD) RATS.

The extended one-generation study design allows the flexibility to investigate a variety of endpoints of interest in animals exposed during critical windows of development. As part of a 2,4-D extended one-generation study which also characterized reproductive toxicity and endocrine toxicity, DNT and DIT were investigated in F1 offspring. An advantage of this design is that stand-alone studies individually examining these endpoints would have used >1500 more animals. CD rats were exposed in utero, during lactation and directly in the diet starting at weaning to 0, 100, 300, 600 (female) or 800 (male) ppm 2, 4-D. PND 22 and 60 offspring showed no exposure-related neuropathology in the central or peripheral nervous system, effects on brain myelin (assessed by special staining) or DNT 60 morphometry. For DNT, 2,4-D had no effect on humoral immune function in males (all dose levels) or females exposed to <300 ppm as measured by antibody plaque forming cell (AFC) responses on PND 70-74. High-dose females had non-significant decreases in AFC/spleen and AFC/106 splenocytes, which appeared to be due to temporal variability over the 4-day evaluation span. This occurred at a dose (600 ppm), which demonstrated non-linear toxicokinetics. For innate cellular immunity, the natural killer cell (NK) assay (PND 87-93) measured target cell cytotoxicity using fluorescent labeled YAC-1 cells (targets) plated with spleen cells (effectors) at E:T ratios from 50:1 to 800:1. The NK assay showed no effects from 2,4-D exposure; linear cytotoxicity with increasing E:T cell ratios was identical across all doses. Thus, there was no evidence of DNT or DIT effects related to 2,4-D exposure.

CHARACTERIZATION OF A NOVEL SERIES OF ENDOTOXIN-RECEPTOR ANTAGONISTS IN THE SETTING OF INFECTION-ASSOCIATED PRETERM BIRTH.

An advantage of the extended one-generation study is that it examines multiple life stages, and offers flexibility to verify age-related sensitivity and/or reproducibility of results. Dietary doses to CD rats were 0, 100, 300, 600 (female) or 800 (male) ppm 2,4-D. P1 males exposed for ~11 weeks showed decreased seminal vesicle and prostate weights (>300 ppm), but there were no associated effects on reproductive function, sperm parameters or pathology. Control relative organ weights exceeded historical control. Decreased testes weights were seen in PND 22 F1 weanlings; 800 ppm F1 males had slightly delayed preputial separation. In both cases, affected offspring had decreased body weights; PND 22 pups showed no associated pathology. Evaluation of groups of F1 males at PND 70 and PND 139 showed no effects on male reproductive organ weights, pathology, and/or sperm parameters. The F1 males, exposed in utero, via lactation and in the diet, did not reproduce P1 organ weight effects, despite longer exposures at higher doses (mg/kg/day). Thyroid hormones (TH), weights and/or pathology were assessed in P1 adults, GD 17 dams, F1 PND 4 and 32 pups, and F1 PND 70 and 139 adults. High-dose GD 17 dams had non-significant TH changes and altered pathology (5 of 12), indicating an adaptive change during the demanding period of gestation. In contrast, TH changes in high- and mid-dose PND 22 pups had no corresponding thyroid weight changes or pathology and were deemed not biologically significant. There were no other effects on endocrine-sensitive endpoints, including anogenital distance or nipple retention, vaginal opening, estrous cyclicity, reproductive indices or litter parameters and mating of a second generation was not triggered. Thus, this integrated study design supports the conclusion that 2,4-D did not induce reproductive toxicity; endocrine toxicity, seen as adaptive thyroid changes on GD 17, only occurred at a nonlinear toxicokinetic dose.

2, 4-DICHLOROPHENOXYACETIC ACID (2, 4-D): EVALUATION OF DEVELOPMENTAL NEUROTOXICITY (DNT) AND DEVELOPMENTAL IMMUNOTOXICITY (DIT) IN A DIETARY EXTENDED ONE-GENERATION STUDY IN CRL:CD(SD) RATS.

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using light microscopy to assess specific organ toxicity. Previous acute toxicity studies in which the animals were sacrificed after 24 hours showed no difference between control and treated animals. From these studies, we can conclude that not only do cadmium at 6 μg Cd2+/L for 14 days result in significant decreases prevent PBT at doses in which we see no toxicity in the mother. It is our hope that these compounds may one day affect the way pregnant women presenting with PTB are treated, as there is currently no FDA approved therapy for this very important clinical disorder.

423 ACUTE TOXICITY OF PISCICIDAL PLANT EXTRACTS (ADENA CISSAMPELOIDEIS) ON TILAPIA (SAROTHERODON GALILAEUS) JUVENILES. S. O. Ayoled and E. K. Ajanii. 1Marine Sciences, University of Lagos, Lagos State, Lagos States, Nigeria and 2Wildlife and Fisheries Management, University of Ibadan, Ibadan, Oyo State, Nigeria. Sponsor: S. Ayoled. The piscicidal quality of water extracts of Adenia cissampeoloides leaves on Tilapia (Sarotherodon galilaeus) juveniles was investigated in a static renewal bioassay to determine the median lethal concentration (LC50) at 96hour of exposure. Five graded concentrations of 800, 600, 400, 200 and 100 mg/litre of aqueous solution of Adenia cissampeoloides and a control 0 mg/10L were applied to S. galilaeus fingerlings in plastic tanks, 1, 2, 3, 4. S & 6 (tank 1 served as control). The 96th LC50 of aqueous extract of Adenia cissampeoloides to S. galilaeus under laboratory conditions was 317 mg/L. Behavioral changes such as erratic swimming, loss of reflex, hyperventilation, increased floating time and circling response for the ET receptor, but these also served prior to death. Histopathological changes in the liver of S. galilaeus juveniles observed are severe widespread vacuolar degeneration and necrosis, hepatocytes, hyperplasia, and presence of large numbers of megacaryocytes. In the gills, there were demudation of gill filaments, swelling of chondrocytes and rarefaction of cartilage within gill filaments. These damages become severe with increasing concentration of the plant extracts.

424 EFFECTS OF CADMIUM ON THE BIOENERGETIC BUDGET AND POPULATION GROWTH OF THE GRASS SHRIMP, PALAEMONETES PUGIO. T. Manyin1, 2 and C. Rowe. 1Environmental Science, SBC (formerly Synasate Research Corporation), Arlingston, VA and 2Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD. Sponsor: J. Carey. Cadmium is a contaminant of concern in the Chesapeake Bay region, where Palaemonetes pugio (grass shrimp) are a key food source for many aquatic species, including commercially important fish species. To determine the effects of aqueous cadmium on the bioenergetic parameters and population growth of grass shrimp, laboratory experiments were performed with subchronic and chronic duration. Exposure to cadmium at 2.1 and 3.8 μg/L DE-71 from early embryo through the completion of metamorphosis. Results from these studies indicated that the rate of metamorphosis decreased with increasing concentration with no marked effect on thyroid gland histology. Measurement of serum thyroxin (T4) and triiodothyronine (T3) were performed during metamorphosis (stages 54 and 58) and at metamorphic climax (stage 61). Thyroid gland T4 was measured at stages 58 and 61. Deiodinase Type II and III (DE-2 and -3) and thyroid hormone receptor beta (TRβ) expression were measured in hind limb (stage 54) and tail (stage 61). Transhyretin (TTR) expression was also measured in liver of stage 58 larvae. Significantly lower levels of both T4 and T3 measured in serum from DE-71 (2.1 and 3.8 μg/L) treated stage 54 larvae, and even more substantially in stages 58 and 61 larvae. However, DE-71 exposure did not significantly alter thyroid gland T4 levels at either stage. A marked decrease in DI-2 expression was noted in hind limb tissue at stage 54 and tail tissue at stage 61 related to DE-71 exposure (2.1 and 3.8 μg/L). A concomitant increase in TTR expression was observed in the 2.1 and 3.8 μg/L DE-71-exposed larvae. Exposure to DE-71 did not alter the expression of DI-3 or TRβ at either stage of development. The combination of decreased DI-2 expression and increased TTR expression suggest a possible mode of action to explain the DE-71-induced reductions in free T3 serum titers at the tissue level and slowed metamorphosis.

425 ELUCIDATING THE MECHANISM OF ACTION OF TRIBUTYLIN (TBT) INDUCED ZEBRAFISH (DANIO RERIO) MASCLINIZATION. C. McGimins and J. F. Crivello. Physiology and Neurobiology, University of Connecticut, Storrs, CT. Xenobiotic compounds can disrupt endocrine signaling, particularly that of steroid receptors and sexual differentiation. The persistent and ubiquitous environmental contaminant, tributyltin chloride (TBT), which was widely used as an antifouling agent can induce masculinization in mollusks. However, only a few studies have addressed the effect of TBT in fish. This study was conducted to investigate the effects of TBT on zebrafish (Danio rerio) masculinization and to determine TBT’s mechanism of action. An expression system was utilized to examine if TBT upregulated or downregulated expression of steroid receptors and expression was quantified by measuring luciferase reporter gene, normalized to protein levels. HeLa cells were transfected with zebrafish steroid receptors, the corresponding response element and treated with 1-100nM TBT. The results show that TBT alone does not activate zebrafish androgen receptor (zAR); however, in the presence of 100nM testosterone, TBT interacts with zAR. Whereas, TBT alone did not effect zebrafish estrogen receptor (zER) expression in the presence of estrogen. Our results suggest that TBT is an indirect androgen activator. Additional studies were performed to analyze which metabolism of testosterone, by their ability to form sulfated (polar) or fatty acid (apolar) conjugates. Sulfation inhibits the biological activity of testosterone, decreasing the affinity for the androgen receptor. While fatty acid conjugation causes testosterone to become apolar, also reducing the metabolic elimination. Female zebrafish injected with 5mg/kg TBT were analyzed for acyl-coa: testosterone acyltransferase activity. Results showed that as TBT concentrations increased, fatty acid conjugated and free testosterone levels in the liver decreased by 60%. Subsequently, in the same TBT injected female fish, liver cytosolic sulfotransferase activity demonstrated a 4 fold increase in sulfated testosterone levels. These results suggest that TBT is exerting its effect on secondary transcription factors, in addition to metabolic elimination pathways.

426 POLYBROMINATED DIPHENYLETHER (DE-71) EFFECTS ON THYROID HORMONE FUNCTION DURING ANURAN METAMORPHOSIS: EVALUATING PERIPHERAL MODES OF ACTION. D. J. Fort1, R. L. Rogers1, P. D. Guiney2 and L. A. Weeks. 1Fort Environmental Laboratories, Stillwater, OK and 2SC Johnson & Son, Racine, WI. An amphibian metamorphosis assay has been proposed by the U.S. EPA in their Tier 1 endocrine screening battery. This work seeks to further validate that assay and examine the mechanisms involved for the brominated flame retardant mixture, DE-71, as it impacts the function of the thyroid axis during metamorphosis in Xenopus tropicalis. A partial lifecycle study was previously conducted exposing X. (Silurana) tropicalis to 0.0, 0.4, 0.9, 2.1, and 3.8 μg/L DE-71 from early embryo through the completion of metamorphosis. Results from these studies indicated that the rate of metamorphosis decreased with increasing concentration with no marked effect on thyroid gland histology. Measurement of serum thyroxin (T4) and triiodothyronine (T3) were performed during metamorphosis (stages 54 and 58) and at metamorphic climax (stage 61). Thyroid gland T4 was measured at stages 58 and 61. Deiodinase Type II and III (DI-2 and -3) and thyroid hormone receptor beta (TRβ) expression were measured in hind limb (stage 54) and tail (stage 61). Transhyretin (TTR) expression was also measured in liver of stage 58 larvae. Significantly lower levels of both T4 and T3 measured in serum from DE-71 (2.1 and 3.8 μg/L) treated stage 54 larvae, and even more substantially in stages 58 and 61 larvae. However, DE-71 exposure did not significantly alter thyroid gland T4 levels at either stage. A marked decrease in DI-2 expression was noted in hind limb tissue at stage 54 and tail tissue at stage 61 related to DE-71 exposure (2.1 and 3.8 μg/L). A concomitant increase in TTR expression was observed in the 2.1 and 3.8 μg/L DE-71-exposed larvae. Exposure to DE-71 did not alter the expression of DI-3 or TRβ at either stage of development. The combination of decreased DI-2 expression and increased TTR expression suggest a possible mode of action to explain the DE-71-induced reductions in free T3 serum titers at the tissue level and slowed metamorphosis.

427 THE EFFECT OF SODIUM TUNGSTATE AND TUNGSTEN ALLOYS ON THE GROWTH OF SELECTED MICROORGANISMS WITH ENVIRONMENTAL AND CLINICAL SIGNIFICANCE. T. L. Doyle and K. L. Mummy. Naval Health Research Center Detachment / Environmental Health Effects Laboratory, Dayton, OH. Sponsor: P. Gunasekher. Tungsten is a transition metal with unique properties that permit its use in a wide range of applications, including household products and small caliber ammunition. Increased use, and therefore exposure, has restored interest in tungsten and tungsten-based products in determining not only their direct impacts upon human health, but also on the environment. Given the critical dependence on microbes for many environmental processes, it is appropriate to evaluate the effect of tungsten on environmental microorganisms, in addition to those that may be relevant to human health. The goal of this study was to investigate the impact of sodium tungstate (Na2W04) and tungsten alloys (W-Ni-Co and W-Ni-Fe) on the growth
of selected microorganisms that are clinically relevant or play key roles in metal reduction, biogeochemical cycling, and biodegradation. In addition, a soil community was also evaluated for its tolerance to Na₂WO₄. To accomplish this, microbial growth was measured for up to three days in nutrient broth or minimal media. Shewanella, a strong metal reducer, displayed the most robust ability to grow even in the highest concentrations of Na₂WO₄ evaluated (250 mM), whereas Pseudomonas putida and Pseudomonas aeruginosa displayed considerably lower tolerances (unable to grow at concentrations >5 mM) and lag phases that increase with Na₂WO₄ concentration (e.g., 3 h in the absence of Na₂WO₄ vs. 16 h at 50 mM Na₂WO₄). Interestingly, bacteria cultivated directly from soil displayed only minor delays and reduction in growth relative to the pure cultures, suggesting that such a microbial consortium is better suited to cope with tungsten exposure. Tungsten alloys also had profound effects on bacterial growth; however, these were highly dependent on the presence of other metals and nutrients, suggesting the effect may be exacerbated in environmental settings where nutrients may be limited.

428 PROTEOMIC SCREENING OF PERFLUOROALKYL ACIDS FOR ESTROGENIC ACTIVITY USING MASS SPECTROMETRY.

M. J. Hemmer1, A. D. Benninghoff2, K. A. Salinas1 and D. E. Williams1. 1Gulf Ecology Division, U.S. Environmental Protection Agency, Gulf Breeze, FL and 2Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.

To evaluate potential estrogenicity of perfluoralkyl acids (PFAAs), two in vivo dietary exposures consisting of a screening study and a dose-response study were conducted with juvenile rainbow trout (Oncorhyncus mykiss). For the screening study, trout were exposed to 10 PFAA compounds (perfluoro-hexanoic [PFHxA], -heptanoic [PFHpA], -octanoic [PFOA], -nonanoic [PFNA], -decanoic [PFDA], -undecanoic [PFUnDA], -dodecanoic [PFDoDA], -tridecanoic [PFTrDA] acids, perfluorooctanoic sulfate [PFOS] and perfluorodecanoic sulfate [PFDS]) at 250 ppm in the diet or a mixture of PFOA, PFNA, PFDA and PFUnDA each at 250 ppm. Fish in the dose-response study were treated with PFOA or PFOS at diet concentrations of 3.2, 16, 80, 400 and 2000 ppm. A single treatment of estradiol (E2) at 5 ppm and solvent control treatments were conducted simultaneously with each 14 day study. Plasma protein profiles were examined using high-throughput matrix assisted laser desorption and ionization time-of-flight mass spectrometry. Estrogen-responsive protein expression models were developed for each study using partial least squares (PLS) discriminate analysis of spectral protein masses from E2-treated and unexposed control trout. Both models produced two significant PLS components with R² values of 0.94 and cross-validated R² values of 0.93. Application of the predictive estrogen model to the screening data set classified 100% of the samples from the mixture treatment as estrogenic. PFNA was the only PFAA tested which showed a positive response where 33% of the samples were classified as estrogenic. Analysis of the PFOA and PFDA dose response samples resulted in classifying 100% of the 2000 ppm PFOA treatment samples and 100% of the 400 and 2000 ppm PFDA treatment samples as demonstrating an estrogenic response profile while the remaining treatments were classified as non-estrogenic. These results support the contention that some PFAAs may act as weak xenoestrogens in teleosts.

429 EFFECTS OF FLUOXETINE (PROZAC) ON AGGRESSIVE BEHAVIOR IN NILE TILAPIA (OERECHROMIS NILOTICUS).


Fluoxetine, a selective serotonin reuptake inhibitor and the active ingredient in Prozac, has been found in effluent run-off and is reported to have a diversity of effects on aquatic vertebrates including decreased survival, induction of developmental abnormalities and altered behavioral patterns. This study examined the effects of fluoxetine on aggressive interactions in male Nile tilapia (Oereochromis niloticus) a species with well described and easy to characterize aggressive behaviors, including lateral display, chase, bite and mouth locking. Individually housed tilapia were presented with an intruder fish in their tanks and aggressive behaviors were scored from video recordings of 10-20 minute trials. In the first series of experiments, tilapia injected with fluoxetine (4mg/kg body weight) showed significant decreases in lateral display, chase, bite and mouth lock behaviors following treatment with fluoxetine. However, in a second set of experiments, tilapia exposed to fluoxetine in the tank water at a concentration of 1.0µg/L (10X the highest environmental concentrations found in the field to date) for a period of seven days showed no significant changes in aggressive behaviors between control and fluoxetine treatments. While environmental exposure to fluoxetine at 10X levels observed in the field did not result in decreased aggression in tilapia it is worth noting that fish exposed to effluent in the field would likely not be exposed only to fluoxetine but to a number of pharmaceuticals and other compounds and that fluoxetine exposure in the field, while variable, might be of greater duration than that examined in this study.

430 A ONE-GENERATION MINK STUDY WITH THREE DIFFERENT DIOXIN AND FURAN CONGENERS.

R. Rasoulpour1, C. Rowlands2, M. Zwiwienik3, J. Moore4, S. Bunian4, D. Kay2 and E. Bursky4. 1The Dow Chemical Company, Midland, MI, 2Entrix Inc., Okemos, MI and 3Michigan State University, East Lansing, MI.

A one-generation reproductive toxicity study in mink was conducted with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); 2,3,4,7,8-pentachlorodibenzofuran (4-PCDF), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); at four dose levels for each congener. Dietary TEQ exposures (TCDD - 2.8 to 13; 4-PCDF – 6 to 23; TCDF – 8.5 to 36 ng/kg/day) occurred over an entire reproductive cycle for 8 to 9 females/treatment group and their offspring. Doses were selected to result in reproductive effects as well as no-effect levels based on previous mink studies. The dosages were also selected to reflect the high end of dietary TEQ exposure of wild mink studied along the Tittabawassee River. A comprehensive set of reproductive and developmental endpoints were evaluated in the adult mink, kits at 6 weeks of age and juvenile mink at 27 weeks of age. TCDD, 4-PCDF or TCDF at their highest doses did not result in statistically significant effects for any reproductive or pup survival endpoints. The mink were not observed in the control or in the TCDD-treated 27-week old juveniles receiving 2.8 ng/kg/day. Minimal jaw lesions (6 out of 10) were observed in 27-week old juvenile mink at the lowest 4-PCDF dosage of 6.2 ng TEQ/kg/day. TCDD at 8.5 ng TEQ/kg/day induced minimal jaw lesions in 1 out of 18 of the 27-week old juveniles. Minimal jaw lesions, therefore, were observed at dietary dosages that exceeded or overlap the high end of the dietary TEQ exposure in wild mink living along the Tittabawassee River.

431 EFFECTS OF TWO ENDOCRINE-ACTIVE PHARMACEUTICALS, TAMOXIFEN AND ANASTROZOLE, ON REPRODUCTION IN A MARINE FISH, TAUTOGOLABRUS ADSPERSUS.


Endocrine-active pharmaceuticals entering the aquatic environment through sewage effluent may have unintended, adverse impacts on the reproduction of aquatic organisms, which in turn may affect the sustainability of exposed populations. Laboratory experiments were conducted with the marine fish cunner (Tautogolabrus adspersus) to evaluate whether the pharmaceuticals, tamoxifen and anastrozole, affected reproduction in spawning adults. Tamoxifen, a widely-used treatment for breast cancer, has been detected in high ng/ml concentrations in British waters. Anastrozole, an aromatase-inhibitor, represents a class of pharmaceuticals that is used to supplement or replace tamoxifen treatment. Aromatase inhibitors are relatively new as a medical option and their concentration has yet to be determined in aquatic environments. The prospects of aromatase inhibitors entering aquatic environments is of concern because aromatase is a key enzyme in estrogen biosynthesis and is critical to normal reproduction. Reproductive endpoints of egg production, egg viability, and egg fertility were assessed daily in spawning cunner treated with tamoxifen (nominal concentrations of 0, 2 or 20 mg/kg) or anastrozole (nominal concentrations of 0.3, 3 or 30 mg/kg) by oral gavage on days 0, 4, 8, 12 and 16 of the experiment. Male and female fish were sacrificed on day 17, and gonadosomatic (GSI) and hepatosomatic index (HSI) were determined. In tamoxifen-treated fish, egg production was significantly reduced (40 - 45%) at both nominal concentrations, while egg fertility and viability exhibited a downward, but not significant, trend. In anastrozole-treated cunner, egg production was significantly decreased (about 35%) in the 3 mg/kg treatment, but no effect was seen on egg fertility or viability in either anastrozole treatment. Results indicate tamoxifen and anastrozole can impact fish reproduction, but further work needs to be done to determine if these effects occur at environmentally-relevant concentrations.
The contamination of aquatic environments with endocrine disruptors (EDs) is highly variable dependent upon source input rates, natural degradation processes and local hydrologic conditions. This creates challenges for environmental monitoring programs, which need frequent sampling to adequately assess the occurrence of ED contamination. Thus, there is potential for underestimating the extent of ED contamination and its impact aquatic organisms. In this study, we monitored four different streams in the greater Puget Sound region of Washington State for the occurrence of estrogenic contaminants and for selective serotonin reuptake inhibitor (SSRI) type pharmaceuticals. We also assessed whether exposure levels were sufficient to impact fish reproduction. Our field sites were selected based on the predominant type of contamination; dairy farm runoff, municipal treated sewage or stormwater runoff. Our experimental design used an integrated approach employing grab samples of water and passive sampling devices deployed for several weeks at each stream site. We also placed caged, sexually mature rainbow trout at each site. The trout were placed in the streams for up to eight weeks and then subsequently spawned to assess fertility and embryo survival. The latter has been demonstrated in past laboratory studies to be the most sensitive toxicological endpoint for freshwater fish. Estrone and estradiol and two SSRIs (fluoxetine and Sertraline) were detected at levels up to 30 PPM. Caged trout at this site also exhibited vitellogenin induction and reproductive failure. The full results of this study will be presented and the significance towards monitoring efforts of endocrine disruptors discussed.

Wastewater effluent and biosolids from municipal wastewater treatment facilities can contain a variety of infectious microorganisms as well as man-made and natural chemical compounds and their metabolites. This can create health risks for both humans and other animals. According to the U.S. EPA and WHO, endocrine disrupting chemicals, such as estrogen and its metabolites, have become a critical emerging environmental concern due to their ability to disrupt normal endocrine function. The objective of this study was to determine the efficacy of high energy (10MeV) electron beam (E-Beam) and chemical oxidants (ferrate and chlorine dioxide) to break down estrogenic compounds in wastewater effluent and biosolids. Wastewater effluent and biosolids samples were collected from wastewater treatment plants. The samples were spiked with 17-β-estradiol and/or treated with six different compounds, as detailed in Table 1. The results of this study suggest that E-Beam (doses ranged from 2 to 12 kGy) and/or treated with different concentrations of ferrate and chlorine dioxide. The estrogenic activity was measured using two different in vitro bioassays, the breast cancer cell line ZR-75 and the YES (Yeast Estrogen Screening) assay. The reduction of estrogenic activity in wastewater after E-Beam irradiation ranged from 71-79% depending on the dose. These results suggest that high dose E-Beam is capable of reducing estrogenic activity associated with wastewater effluent. E-Beam irradiation and chlorine dioxide were less successful at reducing estrogenic activity in biosolids. Ferrate was the only treatment that was able to reduce estrogenic activity in biosolids. Further research is needed to optimize the different treatments and identify the potential end-products of the oxidation process.

Organotin compounds are lipophilic compounds that were once used extensively in the production of antifouling biocides for ships and fishing nets, wood preservatives, agricultural fungicides and pesticides. The widespread uses of these products have resulted in the release of increasing amounts of organotins into the environment, which has proven to be highly toxic to marine organisms. In aquatic invertibrates, particularly marine gastropods, organotin compounds, such as tributyltin (TBT), induce the superimposition of male-type genital organs (penis and vas deferens) on female gastropods (termed imposex), at very low concentrations. The widespread use of these products has resulted in the release of increasing amounts of organotins into the environment, which has proven to be highly toxic to marine organisms. This work shows that AChE activity and MT’s content in collected organisms was not statistically different from that in control organisms. This work shows that AChE activity and MT’s content in collected organisms was not statistically different from that in control organisms. This work shows that AChE activity and MT’s content in collected organisms was not statistically different from that in control organisms. This work shows that AChE activity and MT’s content in collected organisms was not statistically different from that in control organisms.


Organotin compounds are lipophilic compounds that were once used extensively in the production of antifouling biocides for ships and fishing nets, wood preservatives, agricultural fungicides and pesticides. The widespread uses of these products have resulted in the release of increasing amounts of organotins into the environment, which has proven to be highly toxic to marine organisms. In aquatic invertebrates, particularly marine gastropods, organotin compounds, such as tributyltin (TBT), induce the superimposition of male-type genital organs (penis and vas deferens) on female gastropods (termed imposex), at very low concentrations. However, the process of imposex development is not clearly understood. The purpose of this study was to investigate the role of TBT in the masculinization of the female queen conch, Strombus gigas, in the coastal marine environment. Samples of the queen conch were obtained from three sites around the island of Tortola, British Virgin Islands.

Acetylcholinesterase and metallothionein (MT’s) content are biomarkers that indicate the presence of anthropogenic chemicals. Both acetylcholinesterase (AChE) activity and metallothionein (MT’s) content are biomarkers that indicate the presence of anthropogenic chemicals. Both acetylcholinesterase (AChE) activity and metallothionein (MT’s) content are biomarkers that indicate the presence of anthropogenic chemicals. Both acetylcholinesterase (AChE) activity and metallothionein (MT’s) content are biomarkers that indicate the presence of anthropogenic chemicals. Both acetylcholinesterase (AChE) activity and metallothionein (MT’s) content are biomarkers that indicate the presence of anthropogenic chemicals.
In the aquatic environment, pharmaceuticals present unique challenges relative to other contaminants. Unlike other toxicants, drugs are typically designed to have relatively high margins of safety (MOS). For non-drug contaminants the median acute aquatic to Chronic Ratio (ACR) was previously identified at 8 and only 80 at the 90th centile, which coincides with screening approaches using acute testing and default ACRs of 10 or 100. These values may not be protective of aquatic organisms chronically exposed to select therapeutics, where much higher ACR values have been observed (e.g., 176-ethyl estradiol ACR ~2 million). Because of the evolutionary conservation of drug targets among vertebrates, it appears possible to use mammalian data to model fish responses using read-across approaches. One advantage of using mammalian data is the ready availability of acutely toxic and therapeutic (as a chronic effect) dosages. We leveraged mammalian pharmaceutical safety information to explore the potential for effects on fish. We developed a database of mammalian and fish toxicity data for 275 pharmaceuticals. No significant relationship was observed between acute toxicity of fish and mammalian models. We further developed an Acute to Therapeutic Ratio (ATR = LD50/Cmax; similar to fish ACR) and examined these values with a probabilistic pharmacological distribution (PPD), which is similar to a chemical toxicity distribution. ATR values of 12, 3750, and 11,500,000 were identified as the 5th, 50th, and 95th centiles, respectively. Analysis of the ATR PPD showed EE2 (as expected from fish studies) and several other commonly prescribed drugs above the 75th centile. Available fish ACR values were then compared to mammalian ATR values, finding a significant relationship (p<0.05; r²=0.82) between ACRs and ATRs. These efforts suggest that ATRs may provide an approach to understanding potential risks posed to fish from pharmaceuticals in the environment and further supports the use of PPD as a useful tool in screening pharmaceutical hazard to fish.

ASSESSMENT OF METALS CONCENTRATIONS MONITORED IN CARIBOU COLLECTED NEAR A MINING TRANSPORT ROAD IN NORTHWEST ALASKA.

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Caribou are an integral part of life for native northern Alaskans, for both subsistence and cultural reasons. Historical overland transport of ore concentrate from the Red Dog lead/zinc mine in northwest Alaska to the seaport has been associated with elevated concentrations of lead in road soil and on moss growing near the road. Although caribou herds range widely, they sometimes overwinter in areas near the road. Caribou monitoring provides an important component of ecological and subsistence foods monitoring. Thus, a long-term caribou monitoring program was developed in 2009 as part of the comprehensive risk management plan designed to minimize risks associated with fugitive dust emissions from Red Dog Mine. Caribou near the Red Dog Mine have been evaluated for metals concentrations on two previous occasions, in 1996, as part of a region-wide investigation, and again in 2002. The current study reports 2009 monitoring results and evaluates current metals concentrations compared to prior results from the Red Dog mine area and from other areas of northern Alaska without known anthropogenic sources. In spring 2009, 15 caribou were harvested from near the mine and the haul road. Muscle, liver, and kidney tissues were dissected at the time of harvest, frozen immediately, and shipped for metal analysis. Caribou metals concentrations were not consistently higher or lower in 2009 compared to 2002. In comparison with 2002 results, muscle lead (p<0.005), muscle cadmium (p<0.005), and liver cadmium (p<0.05) concentrations were significantly lower in 2009, whereas muscle (p<0.001) and liver zinc (p<0.05) were higher. Lead, cadmium, and zinc from all tissues were within the range of reference concentrations reported for caribou elsewhere in Northern Alaska. These results in conjunction with a multi-pathway health risk assessment completed in 2008 and community blood lead data indicate a) caribou in the area are safe for continued subsistence use and b) the monitoring frequency in the caribou monitoring plan is adequate to verify the continued safety of caribou in the area.
present study was aimed to test our hypothesis that nickel is a minor mass component that may be a major contributor to PM2.5 associated CVD. Methods: Jinchang and Zhangye, two adjacent cities in China, were identified for daily PM2.5 mass and elemental concentrations using XRF. In addition, 30 elderly non-smoking subjects were recruited from each area for a biomarker study. The CVD risk markers, including CRP, MCP-1, IL-6, ICAM-1, and VCAM-1, were assayed using ELISA. Results: Mean ambient concentrations of PM2.5 were 50 and 57 μg/m3 in Jinchang and Zhangye, respectively. However, the ambient level of nickel in Jinchang (216 ng/m3) was 56% higher than that in Zhangye (4 ng/m3). No differences were detected between the two groups in age, BMI, blood pressure, lipid profiles, and blood sugar. CVD risk biomarkers, except ICAM-1, were all higher in subjects recruited from Jinchang compared with those from Zhangye, and significant differences were detected between the two cities only in CRP and IL-6 (p<0.01 and 0.05). Conclusion: Jinchang and Zhangye are unique and ideal environmental settings to examine the roles of nickel in PM2.5 associated CVD. Both CRP and IL-6 may serve as sensitive biomarkers that can be used in the populations of Jinchang and Zhangye to test our hypothesis that nickel is a major component responsible for PM2.5 associated CVD.

442 BIOACTIVATION AND DETOXIFICATION PATHWAYS OF EUGENOL AND METHYLEUGENOL IN HUMAN LUNG AND LIVER MICROSOMES.

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Clove spices are a component of kretek cigarettes and contain significant concentrations of eugenol (CAS 97-53-0). Eugenol is also found in consumer products such as foods and cosmetics. US National Toxicology Program studies found no evidence for eugenol-induced carcino genesis in rats, but equivocal evidence for hepatic tumour formation in female mice. By contrast, the structurally- related compound methyl eugenol (CAS 93-15-2) is consistently carcinogenic. As part of a risk assessment for the use of eugenol in kreteks, we compared bioactivation and detoxification of eugenol and methyl eugenol in human lung and liver microsomes. Pooled human lung and liver microsomes and S9 fractions were incubated with [4C]-eugenol and [14C]-methyl eugenol (20 μm final, 1.8 μl Ci) in the presence of metabolic co-factors. Soluble metabolites were quantified by radio-HPLC and protein-bound metabolites were quantified following extraction with cold acetonitrile. CYP450-dependent formation of the 1-hydroxy genotoxic precursor accounted for 16% and 4% of total eugenol in lung microsomes and S9 fractions respectively. By contrast, the equivalent 1-hydroxy genotoxic precursor accounted for 22% and 30% of total methyl eugenol in the microsomes and S9 fractions respectively. On addition of glucuronide, 85% of eugenol was conjugated and no 1-hydroxy genotoxic precursor was detected. Methyl eugenol was not conjugated under identical experimental conditions. In lung microsomes, formation of the 1-hydroxy metabolite from both eugenol and methyl eugenol was very low (0-4%) and only 5-6% of eugenol was glucuronidated in lung microsomes. Conventional in vitro testing of eugenol metabolites was estimated at 5% in lung and 14% in liver microsomes. Our data demonstrate that methyl eugenol metabolism in the liver leads to accumulation of a 1-hydroxy genotoxic precursor, whereas eugenol bioactivation is limited and glucuronidation is predominant. Lung metabolism is limited for both compounds. These differences may reflect the differing carcinogenic potency between the two compounds.

443 FORMATION OF PHENOL AND HYDROQUINONE METABOLITES OF STYRENE IN MOUSE MICROSONES.

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Styrene is a widely used material in plastic and rubber industry. It has been reported to be hepatotoxic and pneumotoxic in human and rodents. Styrene oxide, the epoxidation product of styrene, was suggested to be the main toxic intermediate. 4-Vinylphenol (4-VP), a ring-oxidation metabolite of styrene, has also been detected in rat and human urine after exposure to styrene and considered another potential toxic metabolite. The main purpose of this study was to seek 2-vinylphenol (2-VP), 3-vinylphenol (3-VP), 2-vinylhydroquinone and hydroquinone metabolites in microsomal incubations. Styrene was incubated with mouse liver or lung microsomes, and the resulting metabolites were analyzed by GC/MS. In addition to styrene glycol (SG) and 4-VP, 2-VP, 3-VP and 2-vinylhydroquinone were detected and verified by the corresponding authentic standards. The production rates of 2-VP, 3-VP, 4-VP, and SG in mouse liver microsomal incubations were 0.0527 ± 0.0045, 0.0019 ± 0.0006, 0.0053 ± 0.0002, and 4.42 ± 0.33 nmol/min/mg protein, respectively. Both disulfiram (100 μm, CYP2E1 inhibitor) and 5-phenyl-1-pentene (5 μm, CYP2F2 and 2E1 inhibitor) significantly inhibited the production of VPs and SG in mouse liver microsomes. 2-VP, 3-VP, and 4-VP were quickly metabolized in mouse hepatic microsome at rates of 2.50 ± 0.30, 2.63 ± 0.13, and 3.45 ± 0.11 nmol/min/mg protein, respectively. The main metabolites of VPs in mice hepatic microsome were 2-vinylhydroquinone, vinylcatechols and hydroxyphenyl ethylene glycol. These metabolites were most prevalent in 2-VP and 3-VP caused similar toxicity in CD-1 mice. In conclusion, 2-VP and 3-VP were identified as phenol metabolites of styrene, and the two phenol metabolites showed mild pneumotoxicity.

444 SPECIES COMPARISON OF IN VITRO METABOLIC CONVERSION OF AFLATOXIN B2 TO AFLATOXIN B1.

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In order to compare the species-different metabolism and susceptibility to toxic and carcinogenic activities of the aflatoxins B2 (AFB2)-to-AFB1 conversion, the liver postmitochondrial fractions were collected from mice, rats, trout, ducks and chickens. These fractions were incubated with [3H]-AFB2 for 15, 30 or 60 min. The amount of [3H]-AFB1 formed from [3H]-AFB2 was determined using high performance liquid chromatography equipped with a flow scintillation analyzer. The AFB2 and AFB1 fractions obtained from thin layer chromatography were confirmed by liquid chromatography/tandem mass spectrometry. The formations of AFB1 after 15 minute incubation with liver postmitochondrial fractions from duck, chicken, rat, mouse and trout were 6.52%, 6.95%, 5.05%, 2.24% and 0.03%, respectively, whereas at 30 minute were 9.22%, 5.86%, 6.03%, 0.66% and 1.09%. The formations were 9.55%, 5.26%, 10.69%, 0.22% and 0.76% after 60 minute incubation. In duck and rat, the formations of AFB1 were much higher than those from other species. This in vitro study of AFB2 metabolism indicates that rat and duck livers are more susceptible to the AFB2-to-AFB1 conversion. Therefore, the toxicity of AFB2 would be greater in rat and duck due to its ability to form AFB1.

445 THE EFFECT OF NEONATAL GENISTEIN EXPOSURE ON AFLATOXIN B1 METABOLISM AND TOXICITY IN RATS.

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In order to study the effects of neonatal exposure to genistein (GS), a phytoestrogen, on the metabolism, acute toxicity and hepatocarcinogenesis of aflatoxin B1 (AFB1) in adult, pups of Fischer 344 rats were injected subcutaneously (SC) with sesame oil or GS (0.08, 0.4, 2 mg/pug) on 2 days of age. Their livers were taken at 2 months of age to study the metabolic activity of the liver toward AFB1. The activity of the postmitochondrial fraction to form AFB1-DNA adduct in vitro was significantly lower in GS treated rats than in control rats. The cytosolic activity to inhibit the in vitro AFB1-DNA adduct formation and the glutathione-S-transferase (GST) activity toward CDN B were significantly higher in GS treated rats than in control rats. The cytosolic enzymes for hepatic functions at 48 hr after AFB1 treatment were significantly lower in GS treated rats than in control rats. The cytosolic activity to inhibit the in vitro AFB1-DNA adduct formation and the glutathione-S-transferase (GST) activity toward CDN B were significantly higher in GS treated rats than in control rats. The cytosolic enzymes for hepatic functions at 48 hr after AFB1 treatment were significantly lower in GS treated rats than in control rats. The cytosolic enzymes for hepatic functions at 48 hr after AFB1 treatment were significantly lower in GS treated rats than in control rats. The cytosolic enzymes for hepatic functions at 48 hr after AFB1 treatment were significantly lower in GS treated rats than in control rats.
Glutathione S-transferases (GSTs; EC2.5.1.18) are a superfamily of multifunctional dimeric enzymes that catalyze the conjugation of glutathione (GSH) to electrophilic chemicals and are the principal enzymes responsible for detoxifying the mycotoxin aflatoxin B1 (AFB1). Commercial turkeys are one of the most susceptible animals known to the effects of the AFB1, a condition associated with efficient cytochromes P450-mediated bioactivation, and deficient GST-mediated detoxification of the active, toxic metabolite the exo-AFB1-8,9-epoxide (AFBO). We have identified and cloned six α-class GST genes from turkey liver and functionally characterized them in an E. coli heterologous expression system. The conserved domains and four signature motifs confirm the α-class identity of the GSTs. His-tag recombinant enzymes (pGSTA1.1, pGSTA1.2, pGSTA1.3, pGSTA2, pGSTA3 and pGSTA4) were purified and their expression levels were confirmed by western immunoblot. GSTs exhibited varying enzymatic activities towards the substrates 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, ethacrynic acid, AFBO, and glutathione peroxidase activity toward cumene hydroperoxide. This genomic approach and biochemical characterization of GSTs was aimed at identifying genetic markers related to AFB1 susceptibility and resistance in turkeys and with the ultimate goal of re-introducing AFB1-protective alleles back into turkeys. Supported in part by NRI competitive grant 2004-35205-14217, from USDA-CSREES, 2006-04819 from the USDA Cooperative State Research, Education, and Extension Service Animal Genome program.

Molecular Cloning and Characterization of Hepatic α-Class Glutathione S-Transferases from Turkeys and Their Role in Aflatoxin B1 Susceptibility.

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Investigation of the Role of Sulfocojugation in Nevirapine-induced Skin Rash.

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Molecular cloning and characterization of hepatic α-class glutathione S-transferases from turkeys and their role in aflatoxin B1 susceptibility.

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Bioactivation of Isoniazid by Hepatic Microsomes.

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Metabolic Interaction of Menthol with Nicotine in In Vitro and In Vivo Test Systems.

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In Vitro Metabolism of 6-2 Fluorotelomer Alcohol in Rat, Mouse, and Human Hepatocytes.


The identification of perfluorinated organic compounds in the environment has generated interest in understanding their biological and environmental fate. 6-2 Fluorotelomer alcohol (6-2 FTOH, CF₃C=CH₂CH₂OH) is a raw material used in the manufacture of fluorotelomer-based products. This study investigated the in vitro metabolism of 6-2 FTOH and selected metabolites by rat, mouse, and human hepatocytes to determine metabolic pathways. Preliminary data showed 6-2 FTOH clearance in hepatocytes from rodent species is more rapid than in humans. Major metabolic pathways for 6-2 FTOH in hepatocytes from all three species included oxidation, hydroxylation, and N-acetylation. The relative amounts of these pathways differed between species, with dehydroepiandrosterone (DHEA) until development of skin rash or for a maximum of 28 days. The levels of NVP, 12-OH-NVP and 12-OH-NVP sulfate were measured in plasma and urine. The incidence of skin rash was 100% among rats treated with NVP or 12-OH-NVP alone and 0–25% for DHEA co-treatment. The plasma concentration of 12-OH-NVP sulfate was 3–7 μg/ml for NVP-treated animals and was decreased to 0.15–2 μg/ml in the co-treated animals. The plasma levels and urinary excretion of NVP and 12-OH-NVP were also decreased and plasma levels of carboxylic acid were increased in the co-treated groups which complicates interpretation of the results.

Conclusions and Discussion: Co-administration of DHEA with NVP to female rats markedly decreased the incidence of skin rash and the plasma levels of 12-OH-NVP sulfate. This suggests that oxidation of 12-OH-NVP to the carboxylic acid can be carried out by CYP 450. The mechanism of skin rash induction by 12-OH-NVP is unknown. We propose that 12-OH-NVP is sulfated in the skin by sulfotransferases leading to a relatively reactive product that binds to proteins in the skin and leads to a rash. The goal of this work was to study the role of 12-OH-NVP sulfate in development of the skin rash by manipulating sulfon conjugation in vivo.

Methods and Results: Female Brown Norway rats were treated with escalating doses of NVP or 12-OH-NVP to 100 mg/kg/day alone or in combination with dehydroepiandrosterone (DHEA) until development of skin rash or for a maximum of 28 days. The levels of NVP, 12-OH-NVP and 12-OH-NVP sulfate were measured in plasma and urine. The incidence of skin rash was 100% among rats treated with NVP or 12-OH-NVP alone and 0–25% for DHEA co-treatment. The plasma concentration of 12-OH-NVP sulfate was 3–7 μg/ml for NVP-treated animals and was decreased to 0.15–2 μg/ml in the co-treated animals. The plasma levels and urinary excretion of NVP and 12-OH-NVP were also decreased and plasma levels of carboxylic acid were increased in the co-treated groups which complicates interpretation of the results.

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HUMAN CYTOCHROME P450 2S1 IS ENABLED TO UTILIZE FATTY ACID HYDROPEROXIDES TO SUPPORT BENZO[A]PYRENE-7, 8-DIHYDRODIOL'S BIOACTIVATION.

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Previously, we showed that a novel dioxin-inducible cytochrome P450, CYP2S1, efficiently metabolizes benzo[a]pyrene-trans-7,8-dihydriodiol (BaP-7,8-diol) into the highly mutagenic and carcinogenic benzo[a]pyrene-7,8,10-trihydriodiol-t-9,10-epoxide (BaP-diol-t-epoxide), using cumene hydroperoxide. Lipid hydroperoxide-supported P450 oxidation has been reported in several cases. However, it has not yet been described for the bioactivation of BaP-7,8-diol. We demonstrate that human CYP2S1 can utilize various fatty acid hydroperoxides to support epoxidation of BaP-7,8-diol at a much higher rate than with cumene hydroperoxide. Kinetic analyses with numerous fatty acid hydroperoxides revealed that 13S-hydroperoxy-9,11-E-octadecadienoic acid (13-HpODE) was the most potent oxidant (Km=3.4±0.8 μM; turnover=0.578 min⁻¹), followed by 12S-hydroperoxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (12-HpETE) (Km=2.8±0.7 μM; turnover=3.76±0.1 min⁻¹), 5S-hydroperoxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HpETE) (Km=2.7±0.8 μM; turnover=3.60±0.09 min⁻¹), and 15S-hydroperoxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (15 HpETE) (Km=11.6±0.8 μM; turnover=0.13±0.03 min⁻¹). Other cytochromes P450, including CYPs 1A1, 1B1, 1A2, and 3A4, were also able to epoxidize BaP-7,8-diol using various fatty acid hydroperoxides, although at slower rates than CYP2S1. The cytoxicity of BaP-7,8-diol significantly increased in mammalian cells expressing CYP2S1 and BaP-diol-t-epoxide formation in these cells also increased in the presence of 13-HpODE. Together, these results suggest that fatty acid hydroperoxides can serve as physiological cofactors in supporting the in vivo CYP2S1-catalyzed oxidation of BaP-7,8-diol, and that fatty acid hydroperoxides and CYP2S1 may play important roles in benzo[a]pyrene-induced carcinogenesis.

CELLS AS A MODEL OF BENZO[A]PYRENE METABOLISM IN NONCANCEROUS HUMAN LUNG: A FUNCTIONAL GENOMICS APPROACH TOWARD UNDERSTANDING METABOLIC CONSEQUENCES OF GENE EXPRESSION.

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Models of carcinogenic metabolism and toxicity in normal epithelium, especially lung, are not well established. In particular, we seek a non-transformed cell system that is likely to reflect the cellular environment for early steps in carcinogenic bioactivation in native lung epithelium. Beas2B cells, an immortal but noncancerous human lung cell line, may serve as such a model. A comparative functional genomics study of basal and induced AKR and P450 expression in Beas2B cells, with implications for metabolic consequences of gene expression, was performed. Real-time PCR analysis of P450 and AKR expression revealed detectable P450A1A and 1B1 mRNA transcripts that were induced after treatment with TCDD. Functional P450 activity, as detected by the EROD assay, was also present. AKR1C transcripts were 100-fold less, and no functional AKR1C activity was detected. Metabolic profiling via radiometric reverse-phase HPLC of 1 μM [1H]-BaP and [1H]-[4]-[Ba]-P-7,8-dihydriodiol was observed in naive and TCDD-induced Beas2B cells. [1H]-BaP metabolism in Beas2B cells resulted in BaP-1,6-dione as the predominant daughter metabolite. [1H]-BaP-7,8-dihydriodiol was metabolized to the four BaP-tetrols, with BaP-tetrol 1 predominant, consistent with P4501B1 expression. Surprisingly however, TCDD was without influence on the metabolic profile of either the parent compound or the dihydriodiol. The AKR product BaP-7,8-dione was not detected, consistent with the low AKR expression. Taken together, these results demonstrate that Beas2B cells are metabolically competent for PAH biotransformation, but do not present evidence for the P450A1A or 1B1 and/or the AKR pathway involved in BaP and BaP-7,8-dihydriodiol metabolism. The detection of BaP-1,6-dione and BaP-tetrol 1 suggests the involvement of peroxidases and/or P450 isoforms in BaP metabolism in this cell line, yet their identity remains elusive. [Supported by IR01-ES015857.]

O-METHYLATION OF PAH CATECHOLS AS A DETOXIFICATION ROUTE FOR PAH O-QUINONES.

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Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants found in tobacco smoke, residues of fossil fuel burning and are associated with the causation of lung cancer. PAH require metabolic activation to elicit their deleterious effects. Cytochrome P450s and aldo-keto reductases (AKRs) are two major activation pathways of PAH trans-dihydriodiol. The formation of redox-active and electrophilic o-quinones catalyzed by AKRs leads to the generation of reactive oxygen species and oxidative damage of DNA. Phase II enzymes can potentially catalyze O-glucuronidation, sulfation and methylation of the PAH catechol and prevent redox-cycling of the PAH o-quinone. We explored whether catechol O-methyltransferase (COMT) could detoxify 7,8-dihydroxy-benzo[a]pyrene formed from the reduction of benzo[a]pyrene-7,8-dione (BPQ). BPQ was reduced to PAH catechol by dithiothreitol under anaerobic conditions and then further O-methylated by bovine COMT in the presence of [3H] S-adenosyl-L- methionine as a methyl group donor. The formation of the O-methylated catechol was detected by both HPLC-RAM-UV and quantified by scintillation counting. Reactions were also replicated using unlabeled cofactor and the O-methylated products were characterized by LC-MS. The kinetics of O-methylation was monitored and a Km of 4.9 μM and Vmax of 3.57 nmol/min/mg was observed using the porcine enzyme. In conclusion, O-methylation is a feasible phase II reaction for the detoxification of PAH o-quinones [Supported by IR01-CA53904 and P30-ES015387].

GREATLY INCREASED SELECTION OF CYTOCHROME P450 ACTIVITIES BY AROCLOR 1254 IN CYP1A2(-/-) AS COMPARED TO WILD-TYPE MICE.

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Dependent upon the extent of the contribution of Cyp1a2 to a monooxygenase activity, it was anticipated that induced activities in Cyp1a2(-/-) mice (breeding stock a grateful gift from D.W. Nebert) would be either no different or to lower levels than in wild-type animals. This outcome was seen following treatment of mice with 3-methylcholanthrene, (20 mg/kg, i.p.) with no differences in induction of 7-ethoxy-4-trifluoromethylquinolinium and 7-ethoxyresorufin (EROD) deethylase activities and lesser induction of 7-benzoxyresorufin (BROD), 7-pentoxyresorufin (PROD) and 7-methoxyresorufin (MROD) dealkylase activities. PROD and MROD activities were markedly lower in naive Cyp1a2(-/-) as compared to naive wild-type mice. Following treatment with Aroclor 1254 (135 mg/kg, i.p.), PROD activity was also induced to a lesser extent in Cyp1a2(-/-) mice, but 7-ethoxy-4-trifluoromethylquinolinium and EROD activities were induced to 2-3-fold higher levels than in wild-type. MROD and BROD showed accelerated induction in Cyp1a2(-/-) animals, with greater induction than wild-type at 48 hours but to similar levels at 120 hours. Induction of total cytochrome P450 and the hypoxochromic shift of the ferrous-carbon monoxide complex at 48 hours were both less in Cyp1a2(-/-) animals than in the reverseases with activities of other P450s, in a manner unrelated to the contribution of the deleted P450 to the activity.

DETOXIFICATION OF ARISTOLOCHIC ACID I BY HUMAN CYP1A2: EVIDENCE FROM HUMAN LIVER MICROSONES.

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Exposure to aristolochic acid (AA) has been identified as the cause of Chinese herb nephropathy (CHN) and a putative risk factor endemic (Balkan) nephropathy (EN). Although much of the related research has focused on the activation of AA to carcinogenic and nephrotoxic metabolites, the detoxification of AA is equally im-

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Tamoxifen is metabolized to metabolites with estrogenic effects but also to reactive intermediates that may form protein and DNA adducts. Both estrogenic and genotoxic mechanisms have been suggested to be responsible for tamoxifen's adverse effects. The cellular expression of tamoxifen-metabolizing enzymes in human extrahepatic organs, such as the intestine, is essential in determining its ability to bioconcentrate in an organism. Therefore, knowledge of the gender differences in the susceptibility to lung injury could also be due to the pro-oxidant role of CYP1B1, which may catalyze the formation of reactive oxygen species, presumably by redox cycling occurring during the metabolism of 17β-estradiol to catecholestrogens. Understanding the role of CYP1B1 in oxygen-mediated lung injury may lead to new strategies to prevent or treat BPD.

Mice deficient in the gene for cytochrome P450 (CYP1B1) are less susceptible to hyperoxic lung injury. Supplemental oxygen therapy is often required to treat premature infants with pulmonary insufficiency. However, prolonged hyperoxic exposures contributes to the development of bronchopulmonary dysplasia (BPD). In this study, we tested the hypothesis that Cyp1b1-null mice will be less susceptible to hyperoxic lung injury than wild type (WT) mice, a phenomenon that would be accompanied by gender differences. Twelve-week-old male and female WT (C57BL/6J) or Cyp1b1-null mice were either exposed to hyperoxia (≥95% O2) or room air for 24-72 hours. Some animals received 4 i.p. injections of either corn oil (CO) or β-naphthoflavone (BNF) (40 mg/kg), prior to hyperoxic exposures. Lung injury was evaluated by lung weight/body weight (LW/BW) and histology. CYP1B1 and CYP1A1/1A2 protein contents and enzyme activities in lung and liver were determined by western blot and fluorimetry, respectively. Cyp1b1-null male and female mice had lesser lung damage than similarly exposed WT mice. WT females showed greater external hyperoxic lung injury than WT males but Cyp1b1-null female mice showed greater decrease in lung damage than males. BNF treatment of WT or Cyp1b1-null mice prior to hyperoxia resulted in decreased lung injury, with Cyp1b1-null mice showing much greater protection in that the lung histology in these animals was similar to those maintained in room air. BNF treatment significantly augmented the pulmonary and hepatic expression of CYP1A1 and 1A2 in both WT and Cyp1b1-null mice. Thus, Cyp1b1-null mice are less susceptible to hyperoxic lung injury than similarly exposed WT mice, presumably due to the pro-oxidant role of CYP1B1 and/or due to augmented expression of CYP1A1/1A2. The gender differences in the susceptibility to lung injury could be due to the pro-oxidant role of CYP1B1, which may catalyze the formation of reactive oxygen species, presumably by redox cycling occurring during the metabolism of 17β-estradiol to catecholestrogens. Understanding the role of CYP1B1 in oxygen-mediated lung injury may lead to new strategies to prevent or treat BPD.

Whether or not bioactivation and covalent binding play a role in drug toxicity is controversial. The first approach for assessing binding potential is microsomal studies with a radioisotope-labeled test article in comparison to benchmark levels. The present study tested the durability of this approach and identified improvements. To assess robustness, an experimental substrate (14C-Compound A) and diclofenac (carbonic anhydrase) were incubated with RLM with or without 3-CHN and EN. (Supported by NIEHS 5P01ES004068 and T32ES007052.)
and M. Colaiacovo.

Knudsen

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C. elegans replicate model of cold sensitivity.

VLCAD (Very Long Chain Acyl-Co-A dehydrogenase) deficiency causes an accumulation of long-chain fatty acids in different tissues. During a period of fasting, children with deficiency may present hypoglycemia, heart problems, muscle weakness and seizures, which can progress quickly to coma and death. In mice, it was observed that VLCAD deficiency leads to abnormal mitochondrial bioenergetics and hypoglycemia, dysfunction, which are more pronounced with fasting or cold stress. We obtained a C. elegans mutant strain lacking the homologue of the enzyme and investigated whether the cold responsive elements in the mouse had similar biological role in the worm. In this study, we used a knockout mutant for k02g10.3, wild type N2, and a transgenic strain (k02g10.3::GFP). All strains were cultured on E.coli at 20°C and synchronized according to standard procedures. Single worm PCR was performed and fluorescent images for GFP and red mitotrackers were obtained using confocal microscopy. For life-span evaluation, 20 worms of each group were transferred to NGM/FUDR plates, placed upon three different temperatures (15, 20 and 25°C) and scored every day. At first, the knockout was confirmed by PCR. We localized the expression of k02g10.3::GFP fluorescence was more intense when compared to worms at 20°C, indicating that this enzyme is active in the cold. Lifespan evaluation supported the involvement of this enzyme in the cold, as KO worms had increased survival compared with N2 or the tg strain. Our data provide evidence that C. elegans can be used to replicate molecular models of cold sensitivity and may help elucidate its molecular mechanisms in disorders of abnormal mitochondrial bioenergetics.
rats, which involves metabolic activation of the toxin through CYP450 enzymes. The nematode Caenorhabditis elegans has emerged as an excellent model for toxicological studies due to its complete genome sequence and cell lineage map, genetic manipulability, and short life cycle. In searching for good alternative models to study toxic mechanisms of environmental toxins, we evaluated toxic effects of AFB1 on development, reproduction, and behavior (movement) of C. elegans. Under non-induced condition, AFB1 concentration up to 15 μg/mL did not cause significant lethal effects (<10% mortality) on adult C. elegans. However, AFB1 strongly disturbed the development, reproduction, and behavior of C. elegans. The juvenile growth was severely suppressed at an AFB1 concentration of 5 μg/mL and above. The reproduction and behavior were also significantly affected with an EC50 of 6.5 μg/mL and 9.5 μg/mL, respectively. Possible toxic mechanism of AFB1 in C. elegans was further investigated by target gene expression screening and analyzing AFB1 metabolites in C. elegans treated with various concentrations of the toxin.

**ZEBRAFISH MAZE BEHAVIORAL ASSAYS.**

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Maze assays using zebrafish and other small freshwater fish are amenable for toxicology screening because of fish robust stereotypical responsiveness, flexibility and efficiency of test design. The active binding sites for neurotransmitters, drugs and toxins are largely conserved among mammals and zebrafish, despite evolutionary divergence. Described here are two behavioral assays that are compliant for neurotoxicity: a fish light-dark plus maze (equivalent to the rodent elevated plus maze) for examining anxiety in a novel environment, and an associative learning (conditioned-place preference) maze in which a food +/- stimulant reward is associated to toxic compounds in the exposure medium. For example, in the aquatic plus maze we found that 3 min bath exposure to the benzodiazepine agonist chlordiazepoxide (5 mg/L), 0.5% ethanol, or 1 week dietary exposure to cannabinoid agonist WIN 55,212 (1 μg/d) zebrafish spent more time in white arms and/or entered white arms more often than controls (ANOVA and Fisher’s LSD p < 0.05, N = 6-8). Nicotine 50 mg/L had no effect on light/dark choice in the aquatic plus-maze, but reduced immobility. Finally, dietary exposure of zebrafish to pesticides dicldrin or chlorpyrifos (10 μg/d) for 2 weeks produced immobility and less time spent in white arms (ANOVA and Fisher’s LSD p < 0.08, N = 4-6). In the associative learning maze, we are investigating the effects of caffeine (50 mg/L) on hungry zebrafish through 5 min exposures before daily conditioning to see if they may acquire association of a food reward with a color (+ purple vs. - green) in fewer rounds than controls. Most of the results presented are the product of student research involvement, and were conducted in adult zebrafish. The mazes can be scaled down in size to accommodate zebrafish larvae or other fish species.

**METABOLIC DEACTIVATION OF ALBENDAZOLE IN THE ZEBRAFISH EMBRYO TOXICITY TEST COMBINED WITH AN EXOGENOUS MAMMALIAN METABOLIZING SYSTEM.**


The zebrafish embryo model for developmental toxicity was recently modified by Busquet et al (2008) by including an exogenous mammalian metabolic activation system (MAS). The purpose was to increase the human relevance by enabling identification of proterogenes that may be activated by mammalian metabolism. The aim of the present study was to test this method with the antihelmintic drug albenzadazole (ABZ), which is teratogenic and has previously been found to form less toxic metabolites. The proterogen cyclophosphamide (CPA) was used as a reference compound. Zebrafish embryos were incubated at 2 hours post fertilization (hpf) with ABZ or CPA and MAS for 60 min at 32°C in tris buffer. For ABZ, which was expected to be deactivated by MAS, the mixture was preincubated for 20 min before embryos were added. The MAS consisted of NADPH (1 mM) and liver micromsomes (0.7 mg/ml) from rat induced with phenobarbital and β-naphthoflavone. After exposure the embryos were placed individually in 96-well plates filled with water. Lethality, developmental delay and malformations were registered at 24 and 48 hpf. Twenty eggs were used for each test group. No abnormalities were observed in vehicle- or MAS alone control groups. CPA (10 μM) induced no embryotoxic effects without MAS but was strongly teratogenic and induced head and tail malformations in the presence of MAS. ABZ was tested at five low doses alone (0.1-1 μM) and at five high doses with MAS (1-27 μM). ABZ alone caused nearly 100% lethality at 0.3 μM whereas an approximately 30 times higher concentration of ABZ was required for the same effect when MAS was present. Chemical analyses of the exposure medium confirmed that ABZ was metabolized by MAS and the deactivation was demonstrated to be NADPH-dependent. We conclude that the method can be used for studying metabolic deactivation of embryotoxic compounds such as ABZ in addition to activation of proembryotoxic compounds such as CPA.

**ZEBRAFISH: A PREDICTIVE MODEL FOR ASSESSING COMPOUND INDUCED NEUROTOXICITY.**

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Zebrafish has been shown to be a useful model for assessing compound induced neurotoxicity. Here we describe methods for validating zebrafish using characterized neurotoxins. We assessed compound effects on motor neurons and neuron proliferation; 5 hour post fertilization (hpf) zebrafish were exposed to ethanol, fixed at 2 days post fertilization (dpf), and stained with both Znp-1, a motor neuron specific antibody and PCNA, an antibody against proliferating cell nuclear antigens. After ethanol treatment, no motor neurons were visible in somites and neuron proliferation was inhibited. To assess compound effects on dopaminergic neurons, 2 dpf zebrafish were treated with 6-hydroxydopamine (6-OHDA), a neurotoxin that destroys catecholaminergic terminals in mammals and then processed for immunostaining with a tyrosine hydroxylase (TH) antibody. Fluorescence signal in TH-positive DA neurons was 44±4% for untreated and 3±4% for treated zebr (P < 0.0001). To assess compound effects on myelin sheaths, we exposed zebrafish to acylamide and observed decreased expression of myelin basic protein in both oligodendrocytes and Schwann cells. Using terminal deoxynucleotidyl transferase dUTP (TUNEL), we determined that L-2 hydroxylglutaric acid (LGA), taxol, acylamide, and a-C jun kinase inhibitor caused brain-specific apoptosis (Parag, Anderson et al 2004; Ton, Lin et al 2006). Fluorescence was quantitated using morphometric image analysis. To confirm presence of BBB, we stained tight junctions in 3 dpf zebrafish using a ZO-1 antibody. We also assessed Evans blue dye diffusion from vessels to the brain at different development stages. In other experiments, using an automated motion detector, we assessed effects of treatment with pentenyleretetrazole (PTZ), a GABA antagonist that induces convulsions in humans, and 4-aminopyridine, amoxapine, methoxsalen, and aminophylline hydrate on zebrafish swim movement; results were similar to results in mammals. Compelling advantages of zebrafish for screening include: transparency, small quantity of drug, convenient drug delivery, short experimental time, low cost, and automation.

**HIGH-THROUGHPUT GENE TRANSFECTION AND RNA INTERFERENCE ON A THREE-DIMENSIONAL (3D) CELL MICROARRAY PLATFORM FOR TOXICOLOGY SCREENING.**

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Recent advances in genomics and proteomics have led to the generation of unprecedented amount of data sets about genes or proteins. Characterizing the in vivo functions of the proteins encoded by tens of thousands of novel genes are exciting but daunting tasks. One approach to identifying the cellular functions of gene products is expressing gene products or inhibiting its synthesis on a microarray platform that is compatible with analyzing thousands of differentially expressed proteins. In the present work, we developed a universal method for transformation of mammalian cells on a microarray chip using recombinant adenoviruses carrying genes of interest for transfection or to silence RNA molecules. Optimization of transfection conditions such as alginate content, cell seeding density, virus concentration, incubation time, and cross contamination by neighboring virus spots were extensively studied with viruses carrying short hairpin (sh) RNAs for loss of function study. Unlike ‘transfected cell microarrays’ developed by the Sahatian group, gene transfection and RNA interference occurred on the miniaturized 3D cell microarrays on a poly(styrene-co-maleic anhydride)-coated slide. Briefly, 20 nl of alginate spots containing mammalian cells was spotted onto 10 nl of alginate spots containing recombinant viruses carrying genes or shRNAs of interest. Spotted viruses were diffused through the alginate gel matrix and transfected mammalian cells, thus allowing the cells to express proteins or to silence RNA molecules. Optimization of transfection conditions such as alginate content, cell seeding density, virus concentration, incubation time, and cross contamination by neighboring virus spots were extensively studied with viruses carrying a gene for GFP or RFP. This cell microarray platform have been applied to express metabolic enzymes and to evaluate the roles of toxic shRNAs for human metabolism and toxicology.
THE USE OF LENS EXPLANT CULTURES TO STUDY THE MECHANISMS OF DRUG-INDUCED CATARACTOGENESIS.

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Treatment with ABC123, a PPAR agonist, resulted in cataracts affecting all treated animals during a 13-week rat repeat-dose study. Lens explant cultures have been described in literature as a reliable model to study the mechanisms of drug-induced cataractogenesis. The present study was conducted to establish this model in-house. Ciglitazone, a PPAR agonist and a cataractogenic compound, was used as a positive control to validate this model. Rat lenses were extracted and cultured in Media 199 supplemented with antibiotics for 24 hrs. Lenses were incubated at 37°C, 21%O2/5%CO2 and 75% humidity. A visual quality assessment was done after 24 hrs pre-incubation and only lenses showing no signs of damage were randomized into four experimental groups, 1) untreated control, 2) 0.1% DMSO control, 3) 10 μM ABC123 and 4) 10 μM ciglitazone (only at 48 hrs). Lenses were treated every 24 hrs after pre-incubation for up to 48 hrs and visually assessed. Samples for viability, histopathology, image analysis and gene expression were collected at 4, 24 and 48 hrs. As compared to the controls there was a time-dependent increase in opacity assessed visually and by image analysis, which correlated to a decrease in viability measured by ATP levels in ABC123 and ciglitazone-treated lenses. ABC123 and ciglitazone had comparable cataractogenic effects after 48 hrs with histology showing rupture of the lens capsule, lens fiber degeneration, cortical lens vacuolation, and lens epithelial degeneration. Gene expression analysis suggested the involvement of early stress response pathways, oxidative and osmotic stress as possible mechanisms of cataract formation. This study confirmed that in vitro lens cultures can be used to elucidate cataractogenic potential of PPAR agonists and to study the underlying molecular mechanisms.

DEVELOPMENT OF A BARRIER-COMPROMISED HUMAN SKIN MODEL FOR IN VITRO PERCUTANEOUS PENETRATION STUDIES.


Procedures for preparing barrier-compromised human skin for use in in vitro percutaneous penetration studies as a model of damaged or disease-state skin, where reduced barrier function due to loss or abnormal development of the stratum corneum (s.c.) is characteristic, were developed. Two methods for disrupting the s.c. were evaluated, namely, tape stripping of the s.c. with 10, 20 or 30 serial tape strips, and topical exposure to sodium lauryl sulfate (SLS) at concentrations of 1, 5 and 10% SLS. The skin samples in the tape stripped groups were tape stripped prior to mounting in diffusion cells, while the skin samples in the SLS treatment groups were mounted in diffusion cells prior to the 4-hour exposures to SLS. A pair of untreated tissues were included as normal skin controls. At least 4 valid trials using human skin from 4 different donors were tested. The barrier integrity of both normal and barrier-compromised skin samples was evaluated by applying an infinite dose of 3H2O topically and evaluating the % of applied dose of 3H2O. The 1, 5, and 10% SLS dilutions induced 8.0, 21.2 and 29.3-fold increases in 3H2O passage relative to the undamaged control tissues (0.179% of applied dose) respectively. While the 10, 20 or 30 serial tape strips induced 3.8, 6.6 and 15.6-fold increases in 3H2O passage relative to the undamaged control tissues (0.179% of applied dose) respectively, the 1, 5, 10% SLS dilutions induced 8.0, 21.2 and 29.3-fold increases in 3H2O passage relative to the undamaged control tissues (0.179% of applied dose) respectively. The present study was conducted to establish this model in-house. The precision of the assay. EpiDerm tissues were treated in duplicate with 0, 1, 3, 10 μg/ml of Mitomycin C, or with 0.1, 0.3, 0.6, or 1.0 μg/ml of vinblastine, in acetone for 48 hours using our standard protocol. Following treatment, single cell suspensions were prepared from the basal cell layers of the EpiDerm tissue constructs, and cells were processed for flow cytometry using the Litron In Vitro MicroFlow® kit. Single cell suspensions from tissues treated in parallel with cytochalasin B and the same concentrations of mitomycin C or vinblastine were analyzed.

The Reconstructed Skin Micronucleus Assay (RSMA) using EpiDerm™ tissues (MatTek Corporation) has been developed as a possible replacement for in vivo genotoxicity testing of cosmetics, which is now banned in Europe by the Seventh Amendment to the Cosmetics Directive. The assay is currently undergoing evaluation for interlaboratory reproducibility in the United States and Europe as part of an international validation effort. The assay performs well for correctly identifying positive and negative genotoxins, but scoring micronucleus induction microscopically is extremely labor intensive, which may limit widespread use of the assay. We have demonstrated that the feather follicle is a good, reliable model for assessing the precision of the assay. EpiDerm tissues were treated in duplicate with 0.1, 3, 10 or 30 μg/ml of Mitomycin C, or with 0.1, 0.3, 0.6, or 1.0 μg/ml of vinblastine, in acetone for 48 hours using our standard protocol. Following treatment, single cell suspensions were prepared from the basal cell layers of the EpiDerm™ tissue constructs, and cells were processed for flow cytometry using the Litron In Vitro MicroFlow® kit. Single cell suspensions from tissues treated in parallel with cytochalasin B and the same concentrations of mitomycin C or vinblastine were analyzed. The results from In Vitro MicroFlow® analysis were comparable to those from standard microscopic scoring, demonstrating that this automated scoring platform can be used to quantify genetic damage in a 3-dimensional epidermal tissue construct. Since flow cytometry is already widely used by many laboratories and its efficiency and objectivity are well known, this development has the potential to increase the usability of the RSMA. The Reconstructed Skin Micronucleus Assay (RSMA) using EpiDerm™ tissues (MatTek Corporation) has been developed as a possible replacement for in vivo genotoxicity testing of cosmetics, which is now banned in Europe by the Seventh Amendment to the Cosmetics Directive. The assay is currently undergoing evaluation for interlaboratory reproducibility in the United States and Europe as part of an international validation effort. The assay performs well for correctly identifying positive and negative genotoxins, but scoring micronucleus induction microscopically is extremely labor intensive, which may limit widespread use of the assay. We have demonstrated that the feather follicle is a good, reliable model for assessing the precision of the assay. EpiDerm tissues were treated in duplicate with 0.1, 3, 10 or 30 μg/ml of Mitomycin C, or with 0.1, 0.3, 0.6, or 1.0 μg/ml of vinblastine, in acetone for 48 hours using our standard protocol. Following treatment, single cell suspensions were prepared from the basal cell layers of the EpiDerm™ tissue constructs, and cells were processed for flow cytometry using the Litron In Vitro MicroFlow® kit. Single cell suspensions from tissues treated in parallel with cytochalasin B and the same concentrations of mitomycin C or vinblastine were analyzed. The results from In Vitro MicroFlow® analysis were comparable to those from standard microscopic scoring, demonstrating that this automated scoring platform can be used to quantify genetic damage in a 3-dimensional epidermal tissue construct. Since flow cytometry is already widely used by many laboratories and its efficiency and objectivity are well known, this development has the potential to increase the usability of the RSMA. Making the RSMA widely accessible to laboratories throughout the world is an important step for the acceptability of this assay for regulatory use.
The Bovine Corneal Opacity and Permeability assay (BCOP), an internationally recognized alternate to the Draize eye irritation test, uses excised bovine corneas to predict ocular irritation. Originally developed by Gautheron (1992) and utilizing the irritation class prediction established by Sina (1994), BCOP has been used independently at MB Research and at the Institute for in Vitro Sciences (IVIS) for over fifteen years for product development, worker safety, and safety claims substantiation. The assay has recently been included in an 18-month pilot evaluation program for use for eye irritation labeling of cleaning products with antimicrobial claims (EPA Office of Pesticide Programs, May 2009). In addition, the Organization for Economic Co-Operation and Development (OECD) adopted Test Guideline 437 describing the use of the assay for identifying ocular corrosives and severe irritants (Sept. 2009). Since MB Research and IVS have extensive experience performing the BCOP assay utilizing a variety of protocols, they agreed to develop and evaluate the reproducibility of a standardized protocol for regulatory labeling. Nine blind-coded chemicals, primarily comprised of surfactant dilutions, as well as a standard control, were tested in three independent IP-compliant trials using exactly the same protocol. The resulting In Vitro Scores were compared to Draize MMAS results (ECETOC, 1998). Intra-laboratory and inter-laboratory reproducibility evaluations showed that both laboratories obtained the same irritation class predictions (except for ceteryl pyridinium bromide). Some of the surfactant dilutions (sodium dodecyl sulfate, ceteryl pyridinium bromide) were found to be under-predicted using the standard BCOP protocol for liquid test chemicals. Accordingly, the testing of certain classes of surfactants for regulatory safety using extended exposure times may be scientifically justified.

There is much interest in the development of in vitro tests for the identification of chemical allergens, particularly those using dendritic cell (DC) and DC-like cells such as the THP-1 monocytic cell line. Limitations of such methods include effective delivery of chemicals to aqueous culture, toxicity and narrow dynamic range. The impact of growing cells on ECM rather than in aqueous culture has now been examined. This approach has the potential benefit that preconditioning ECM with chemical allergen may provide an alternative allergen delivery system. Human keratinocyte (HaCaT) and human fibroblast (TIF) cell lines were used to produce epidermal and dermal ECM, respectively. Cells were grown on glass cover slips for 7 days and denuded of cells; control cover slips were coated with polylysine-D (PD). THP-1 cells (2x10^5 cells/ml) were cultured for 24h on ECM- or PD-coated cover slips or in aqueous suspension in the presence of 0-100μM of the contact sensitizer 2,4-dinitrochlorobenzene (DNCB). Cell viability was assessed by flow cytometry and interleukin (IL)-8 production by ELISA. Similar dose-dependent effects on viability were recorded for all culture conditions, with <200 μM DNCB. Higher basal levels of IL-8 were observed following culture on ECM- or PD-coated cover slips compared with cell suspensions. However, a similar pattern of DNCB-induced changes in IL-8 secretion was seen for all treatments, with enhanced IL-8 expression recorded for 5μM DNCB (a subtoxic dose), whereas higher doses reduced IL-8 secretion. Preconditioning of ECM-, but not PD-, coated cover slips with DNCB for 4h resulted in increased responses, with doses of 100-1000μM of DNCB up-regulating IL-8 expression without concomitant loss in viability. These results suggest that culture of THP-1 cells on ECM improves the dynamic range of responses to DNCB. It may be possible to exploit this phenomenon to improve the sensitivity of in vitro tests for the identification of skin sensitizers.
and D. R. Cer Corporation, Ashland, MA and MatTek in vitro Life Sciences Laboratories, Brezniceva, Slovakia.

Determination of skin irritation potential is an international regulatory requirement to ensure safe handling, packaging, labeling, use and transport of chemicals, cosmetics and household products. Recent REACH legislation and a ban on animal testing for cosmetics have heightened needs for validated in vitro Skin Irritation Tests (SITs). A UN treaty endorsed by the US, EU, China, Japan, Australia and others has outlined a Globally Harmonized System of Classification and Labeling of Chemicals. The GHS classifies skin irritation of chemicals into three categories: non-irritant (NI or no label), slight irritant (SI or class 3) or irritant (I or class 2). The EpiDerm model has been validated for in vitro skin corrosion testing endorsed by OECD as Test Guideline (TG) 431. An OECD TG for in vitro SIT based on 3 ECMAM validation studies is under way. However, currently validated SITs distinguish only 2 classifications – Class 2 and no label, thus they do not completely satisfy needs of regulatory bodies requiring 3 classifications. Therefore, additional efforts are underway to validate an EpiDerm SIT for GHS. Previous work utilizing 15 reference chemicals established a preliminary EpiDerm-GHS-SIT prediction model (The EpiDerm, 108(1):379-390). Here we report results with an expanded set of 36 chemicals including 12 from each GHS category. Using a tiered strategy with 2 MTT viability assay protocols, SI plus I chemicals were classified from by-product abattoir eyes. Test articles (liquid and solid) are dosed directly onto the ocular surface, and tissue damage and recovery are assessed by sodium fluorescein (NaFl) retention in the same cornea over time (up to 21 days). We have confirmed NaFl retention results and corneal recovery in the PorCORA system via several approaches. Both fluorescence and reflective confocal microscopy confirm damage repair indicated by fluorescein retention in the cultured corneas. In addition, we have shown histological evidence that also correlates well with NaFl staining in the PorCORA assay. Here we report the results of a 32-reference chemical validation including chemicals from the following classes: acetates, acids, alcohols, alkalins, esters, hydrocarbons, inorganics, ketones, surfactants, and several solid compounds. To determine if the PorCORA system can predict R41 or GHS Category 1, we considered corneas that retained NaFl at 21 days post-dose to be R41 and GHS Category 1. ECETOC historical rabbit eye data was used to classify EU and GHS eye irritation for the 32 compounds tested. PorCORA predicted 11/11 compounds classified as R41 and 12/13 compounds classified as GHS Category 1. Since PorCORA can predict these categories, then compounds that cause damage that is reversible in the PorCORA system may be considered R43 or Category 2. Thus PorCORA is a highly predictive method to distinguish between ocular irritancy classifications R36 or R41 and Category 1 or 2 without the use of live animals.

480 ESR EVIDENCES OF NO2 DERIVED PEROXYNITRITE AS TRIGGERING NEWER DIESEL ENGINE EMISSION LUNG OXIDANT INJURY. OXIDATION CATALYSIS RESPONSIBILITY.

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Diesel particulate matter has frequently been claimed as the main trigger but some studies suggest that gas phase pollutant might be responsible of oxidative stress induced health effects. NO2 could be one of the major potential triggers. A continuous flow exposure device to continuously sampled and diluted engine exhausts using a biphasic air/liquid culture of rat lung tissue is used. Physical properties of the aerosol and pollutant bioavailability remain unaltered in the delivered aerosol. Study of the impact of treating Diesel exhaust by oxidation catalysis and diesel particulate filter on lung tissue oxidative stress and reactive oxygen species production measured by electron spin resonance was conducted. ESR measurement using CPH as a spin probe was also measured in exposed culture media to a continuous flow of aerosol in the absence of lung tissue and proved to be very useful for assessing the oxidant potential of the aerosol. A highly significant correlation between NO2 concentrations in the aerosol and the ESR signal was suggestive of a potential major role of NO2 compared to Diesel soot for triggering Aerosol filtration for removing soot did not alter the ESR signal from the aerosol. Diesel exhaust induced oxidative stress was identified. To verify the potential role of NO2, synthetic NO2 was delivered from a cylinder and a series of antioxidants was tested for their ability to prevent the formation of ROS assayed by ESR. It is demonstrated that MEG (a peroxinitrite scavenger), prevents the occurrence of ROS production upon NO2 exposure. Glutathione, ascorbate and sodium dithionite exhibited similar activities as MEG. Diesel exhaust treated by oxidation catalysis induced major oxidative stress and lung tissue glutathione depletion, compared to untreated emissions. Evidence that NO2 might trigger these effects through the formation of peroxinitrite which is known to be detrimental to biological tissues especially to lung and heart are discussed.

479 PORCINE CORNEAL OCULAR REVERSIBILITY ASSAY (PORCORA) PREDICTS EU R41 AND GHS CATEGORY 1.

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Currently, there is no alternative (non-in vivo) ocular irritation assay that can measure cornal tissue damage and reversibility. With the support of two Colgate-Palmolive Grants for Alternative Research, we have developed an alternative assay: Porcine Corneal Opacity Reversibility Assay (PorCORA). PorCORA measures corneal damage and recovery over extended time periods using porcine corneas excised from by-product abattoir eyes. Test articles (liquid and solid) are dosed directly onto the corneal surface, and tissue damage and recovery are assessed by sodium fluorescein (NaFl) retention in the same cornea over time (up to 21 days). We have confirmed NaFl retention results and corneal recovery in the PorCORA system via several approaches. Both fluorescence and reflective confocal microscopy confirm damage repair indicated by fluorescein retention in the cultured corneas. In addition, we have shown histological evidence that also correlates well with NaFl staining in the PorCORA assay. Here we report the results of a 32-reference chemical validation including chemicals from the following classes: acetates, acids, alcohols, alkalins, esters, hydrocarbons, inorganics, ketones, surfactants, and several solid compounds. To determine if the PorCORA system can predict R41 or GHS Category 1, we considered corneas that retained NaFl at 21 days post-dose to be R41 and GHS Category 1. ECETOC historical rabbit eye data was used to classify EU and GHS eye irritation for the 32 compounds tested. PorCORA predicted 11/11 compounds classified as R41 and 12/13 compounds classified as GHS Category 1. Since PorCORA can predict these categories, then compounds that
Abnormal neuronal development has been observed in D3 and MCT8 null mice demonstrating the importance of tightly regulated intracellular thyroid hormone (TH) concentrations. We have studied ENeSt-A human neural stem cells as a model to study cellular TH metabolism in human neurogenesis. mRNA expression of TH regulatory proteins during differentiation showed marked up-regulation of D3, the enzyme inactivating T4 and T3, and down-regulation of MCT8, a thyroid hormone transporter. In this study, immunofluorescence showed co-localization of D3 (anti-D3 antibody courtesy of Dr. T.J. Visser) with neuronal marker β-tubulin III (BTIII). Neuroprogenitors (NP) and differentiating neurons (DN) were incubated with 125I-T3 followed by metabolic analysis by HPLC. D3 enzymatic capacity was high: even at free T3 (fT3) concentrations 8000-fold of euthyroid fetal fluids, fractional T3 deiodination was reduced only slightly in NP, and was actually increased in DN. Deiodinase inhibitor iopanoic acid (IOP) blocked T3 degradation allowing significant accumulation of intracellular [125I]-T3 in DN. Phenotypic outcome was quantified in 96-well plates by immunofluorescence using neural stem cell marker nestin and BTIII. No significant alteration in proliferation rate was observed from 0.2-μM fT3, yet synaptophysin mRNA, a synaptic-nexin marker, was highest at 0.1 μM fT3. Significant impairment of NP proliferation was observed at fT3 concentrations of 20 and 200 μM. IOP decreased BTIII immunofluorescence in DN when cells were differentiated in 6 μM fT3. No change in BTIII mRNA in DN was observed, suggesting a post-transcriptional effect of T3. In conclusion, TH deficiency impacted neither neuroprogenitor proliferation nor differentiation, but excess T3 or inhibited D3 activity perpetuated undifferentiated nestin-positive cells and impaired outgrowth of differentiating neurons. We postulate that D3 may be an as yet unrecognized target for thyroid disruptors leading to altered neuronal development.

**483 EPIDERM FULL THICKNESS SKIN CULTURES (EFT) AS AN IN VITRO MODEL FOR WOUND HEALING.**

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Background: Wound healing is a natural process, which involves the regeneration of epidermal and dermal tissue with expression of growth factors, cytokines and chemokines. The objective of this study was to evaluate the EpiDerm full thickness (EpiDerm-FT, EFT) skin culture as an in vitro wound healing model to understand the healing process of chemical and burn wound. Methods: A strong base 32N KOH, and 2 strong acids, concentrated H2SO4 and 100% Acrylic acid (AA), were selected for making chemical wounds and a heated 2 gram standard weight made of brass was used for making significant burn wound. Initially, the dose (chemical) and exposure time required to create a significant wound was optimized. Immediately after inducing a wound, cultures were washed thoroughly with phosphate buffered saline (PBS) and incubated at 37°C. The culture medium and EFT tissues were collected every alternate day until the 6th day for histological studies, and to study the release of growth factors, cytokines and chemokines. Results: Histological sections of 32N KOH treated cultures clearly showed damage to stratum corneum and upper layers of epidermis and no damage to dermis. However concentrated H2SO4 produced a deeper wound in the epidermis without significant damage to the stratum corneum. Acrylic acid only damaged the dermis but did not appear to damage the stratum corneum or epidermis. In the case of burn wounding, the 2 gram weight-induced burn showed significant damage to all layers of EFT tissues when exposed for 15 seconds. Histological sections showed regeneration of epidermis and dermis in both types of wound. Further, increase in release of growth factors like Collagen IV & Epidermal Growth Factor as demonstrated by western blotting and immunohistochemistry suggested wound healing. Of growth factors like Collagen IV & Epidermal Growth Factor as demonstrated by western blotting and immunohistochemistry suggested wound healing.

**484 ACTIVATION OF PROTEASE-ACTIVATED RECEPTOR-2 BY SERINE PROTEASE ENZYMES STIMULATES MCSF RELEASE FROM HUMAN RESPIRATORY EPITHELIAL CELLS.**

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Airway epithelial cell response to allergen is a first step to mounting a Th2 immune response through release of Th2 cell-promoting cytokines. We showed that Alcalase® (Bacillus serine protease and occupational respiratory allergen) induced a dose-dependent production of the inflammatory cytokine, MCsf, from human airway type II-like bronchial epithelial cell line A549 grown in an air/liquid interface. In this study we examined the role of protease-activated receptor (PAR) and NFκB in this response. Using 3 PAR activating peptides for PAR1, PAR2 and PAR4 and trespin, a known agonist for PAR2, we showed that activation of PAR-2 on A549 cells induced M-CSF and IL8 secretion. Inhibition of NFκB pathway with BAY 11-7085, an NFκB pathway specific inhibitor, had a marginal effect on M-CSF production but it completely blocked IL-8 secretion. Since PAR-2 signaling is via NFκB then Alcalase induced MCsf may work through multiple pathways. MCSF enhanced the surface expression of the adhesion molecule CD54 and the maturation marker CD83 on the human monocytic leukemia cell line THP-1, which has the potential to differentiate into dendritic cells. Therefore, MCsf may serve as a specific indicator of an initial allergic response and contribute to the induction of the Th2 immune response by proteases.

**485 PORCORA OCULAR REVERSIBILITY ASSAY TESTING WITH PERSONAL CARE PRODUCTS.**

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To ensure consumer safety, ocular irritation testing is routinely performed on personal care products. Two alternative ocular toxicity tests, the Chorioloiontio Membrane Vascular Assay (CAMVA) and Bovine Corneal Opacity and Permeability Assay (BCOP), are widely used in the cosmetic industry since they do not involve the use of animal. These assays provide ocular irritation assessment and have high predictive value when compared to rabbit or human eye results. To complement the CAMVA/BCOP assays, the Porcine Corneal Opacity Reversibility Assay (PorCORA) was developed using an ex vivo model to predict reversibility of ocular damage caused by potential irritants. In the current study, three commercially available consumer products (a shampoo, a hair color glaze, and a 12% hydrogen peroxide product) were tested in the PorCORA for ocular damage and reversibility. The PorCORA indicates that under the exaggerated in vivo study conditions the surfactant-based shampoo may cause irreversible ocular damage; histological changes occurred in the squamous-cell layer of the corneas and mild to moderate changes in the basal-cell layer. However, scientific literature contradicts these results, and ocular damage reversibility does occur in vivo following exposure to shampoo. Furthermore, the PorCORA predicts that under the same study conditions used for the shampoo, ocular damage caused by a hair color glaze and a 12% hydrogen peroxide product are fully reversible with histology reporting only minimal or mild microscopic effects to the superficial squamous-cell layer. Like the shampoo, the scientific literature also indicates that reversibility of ocular damage occurs in vivo following exposure to hydrogen peroxide. In summary, the PorCORA assay, in conjunction with other alternative toxicity ocular irritation assays is a valuable and predictive method to determine the extent of ocular damage and reversibility that products may cause following consumer eye exposure.
Highly differentiated, organotypic tissue models are being used increasingly in lieu of animals to meet regulatory testing requirements. The reproducibility of these tissue models is of prime importance so that US and EU regulators and industry can be assured that the toxicological system is reproducible both during the validation process and afterwards. The purpose of this study was to investigate the effects of various tissue culture inserts (TCI) on the tissue morphology and reproducibility. Tissue culture inserts (TCI) from 5 commercial manufacturers were obtained and standardized culture conditions were used to produce the skin (EpidermTM) and ocular (EpisoratocTM) organotypic tissue models. These tissue models were then subjected to quality control (QC) tests which include histological evaluation and determination of the exposure time of a common surfactant (Triton X-100) that reduces the tissue viability to 50% (ET-50). Of the 5 TCI tested, tissue histology for tissues cultured on 2 TCI was distinctly different and inferior to the standard tissues while the histology for tissues produced on the remaining TCI were structurally equivalent to the control tissues. The average ET-50 for Epiderm produced with the best substitute TCI was 7.64 +/- 0.95 hr (n=6) and was not statistically different than that of tissues cultured on the control TCI, 7.73 +/- 0.56 hr (p = 0.79, paired student t-test); similarly, the ET-50 for Episoratoc cultured on the best alternative TCI was 22.6 +/- 5.0 min (n=5), which was not statistically different than that of the control tissue, 27.2 +/- 3.0 min (p = 0.18, paired student t-test). In summary, the TCI is one of the crucial parameters in producing high quality, reproducible, organotypic tissue models but multiple commercially available TCI appear to have appropriate properties.

The in vitro SkinEthicTM reconstructed Human Corneal Epithelium (HCE) is part of an on-going ECVM formal in vitro eye irritation validation study aiming a possible incorporation of the test method in a tiered test strategy to replace the Draize eye test (OECD TG 405). The test method, based on tissue viability measurement by using the MTT assay, was specifically designed to allow the discrimination between irritant and non-irritant substances. The reduced MTT (purple formazan) is quantified by a standard colorimetric method. During formazan extraction, any unspecific color remaining in the tissue or developed in situ may be extracted and consequently induce a possible final viability overestimation. In this study specific controls were introduced allowing the use of the test method for the irritation prediction of colored/coloring substances. The protocol consisted mainly in a short 10 minutes topical treatment or a long 1h +16 hrs post-treatment incubation period. After rinsing, some colored substances retained in the epithelium can induce a residual staining. Unspecific color, was quantified by using treated tissue controls following the standard protocol course but not exposed to MTT. These controls enabled the quantification of Non Specific Optical Density (NSOD) and the correction of final measurements (true OD due to mitochondrial activity). Histological analysis can be conducted in order to document strong coloring substances (+ 30% relative to negative controls). In this study, 9 irritants and 10 NC colorants have been evaluated following this strategy. We showed that the SkinEthicTM-HCE assay is a suitable method for the in vitro eye irritation prediction of coloring substances. The applicability domain of this assay can therefore be extended to these substance families.

The need for alternatives to animal based skin sensitization testing has spurred research on the use of in-vitro, in-silico and in chemical methods. Gluthione and other select peptides have been used to determine the reactivity of electrophilic allergens to nucleophiles but available peptide-based methods are inadequate to accurately measure the rapid kinetics observed with many chemical sensitizers. A kinetic spectrophotometric chemometric assay involving the reactivity of electrophilic sensitizers with nitrobenzenethiol was evaluated. Stopped flow techniques and conventional UV spectrophotometric measurements enabled determination of reaction rate constants ranging from 6 x 10-3 M-1s-1 to 2.7 x 104 M-1s-1. Rate constants were measured for 3 extreme, 5 strong, 3 moderate and 2 non-sensitizers. Nine of 13 and 2/3 predicted skin sensitizers exhibited pseudo-first order and second order kinetics, respectively. In 2/3 chemicals deviations from first and second order were apparent where the chemicals exhibited complex, likely mixed order kinetics. The reaction rates of the electrophiles correlated positively with their EC3 values within the same mechanistic domain. Detailed specific chemical knowledge of the test chemical is of paramount importance, since false negatives were observed with sensitizers like trimellitic anhydride and otoxane. Findings from this model show that for the same mechanistic domain, skin sensitization is driven mainly by electrophilic reactivity. This simple and rapid absorbance based method can be incorporated into the battery of alternative non-animal assays for use in skin sensitization prediction.

A common goal of many personal care companies is to assure the safety of their products without animal testing, due to concerns about ethical and animal welfare issues as well as the relevancy of the animal model to humans. To address these issues, we have developed an in vitro testing program to support the safety evaluation of potential vaginal irritation in a number of bath and shower cleanser products. A series of surfactant-containing formulations, diluted to 10% in water to mimic the maximum concentration expected in bath water, were tested. The formulations were applied topically onto the surface of commercially-available SkinEthic HVE three-dimensional human vaginal epithelium tissues over various exposure times. The ET50 (i.e. the exposure time expected to reduce relative viability of the tissues to 50% of controls) for each candidate was determined. The test results were compared to reference formulations and available human clinical data. The vaginal irrit-
tancy evaluation and ranking of bath and body wash products based on ET50 values showed a good correlation with the expected irritation potential of individual ingrediants. Histology analysis confirmed the overall MTM viability results and pro-
vided additional information regarding the effect of the tested products on the tis-
sues integrity. However, IL-1β release did not appear to be as sensitive a marker as the MTM viability assessment at the short exposure times used (20 minutes, 1, 2, and 4 hours). This in vitro safety screening approach shows promise for predicting the vaginal irritancy of tested products and in meeting the typical needs of product development groups charged with developing increasingly milder products.

**492 DEVELOPMENT AND CHARACTERIZATION OF HUMAN AND MOUSE PRIMARY EPITHELIAL CELL CULTURES FOR ASSESSING ARSENIC TOXICITY.**


Inorganic arsenic exposure can induce skin, lung, and bladder cancer in humans; however, it has typically not produced tumors in standard animal bioassays. To bet-
ter understand the mechanisms of arsenic induced bladder cancer and the reasons for species differences in sensitivity, in vitro tissue and species specific models are needed. The purpose of this study was to develop human and mouse primary uroepithelial cell culture models to investigate arsenic toxicity of the bladder. Human ureter sections from transplant patients were received in refrigerated Custodiol® solution. Epithelial cells were isolated by gently scraping the interior to release the cells. Cells were passed through a 100 micron nylon filter, centrifuged, and washed with keratinocyte-SFM to remove debris. Cell viability was >80%. Cell yield varied depending on the size and quality of donor tissue, but average yield was 3.7 × 10^6 viable cells. Human cells were seeded at 50K/well in collagen-coated 96-
well plates and grown to confluency for ~3 weeks. RT-PCR analysis confirmed PPAR-gamma, Keratin 10 and UPK2 expression, indicating cell differentiation and proliferation. Mouse uroepithelial (UE) cells were isolated using the same protocol as human ureter cells. Total cell yield was approximately 0.5 × 10^6 cells per bladder and cells were seeded at 100-200K per well in collagen-coated, 96-well plates. Unlike human cells, mouse cells did not adhere to the culture plates and did not ex-
press key markers of growth and differentiation. Cell viability decreased rapidly over time. Several variations in media and plate coatings did not improve mouse cell viability or growth properties. This work demonstrates that human ureter ep-
ithelial cells may be useful for assessing arsenic toxicity and improving risk assess-
ment models, while the use of mouse UE cells requires additional work.

**493 DERMAL ABSORPTION IN RATS EXPOSED BODY-
ONLY AND NOSE-ONLY TO CHEMICAL VAPORS.**

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Groups of 6 rats each were exposed body-only or nose-only for 4 hours to vapor atmo-
ospheres of 4 chemicals: toluene, isopropanol, dioxane, or methyl ethyl ketone (MEK). Blood samples were collected from each rat at 0, 1, 4, and 18-hour time-
points and analyzed for concentration of the primary chemical and its metabolites. The mean blood concentration of toluene in rats exposed to 8,000 ppm toluene ranged from 12-13 μg/ml and 210-240 μg/ml for rats exposed body-only and nose-
only, respectively. The mean blood concentration of isopropanol/acetone in rats ex-
posed to 10,000 ppm isopropanol ranged from 2-4/15-32 μg/ml and 410-
1200/240-980 μg/ml for rats exposed body-only and nose-only, respectively. The mean blood concentration of dioxane in rats exposed to 5,000 ppm dioxane ranged from 8-33 μg/ml and 430-1700 μg/ml for rats exposed body-only and nose-only, respectively. The mean blood concentration of MEK/2,3-butanediol in rats ex-
posed to 5,000 ppm MEK ranged from 5-20/3-5 μg/ml and 430-1700 μg/ml for rats exposed body-only and nose-only, respectively. The concentration of chem-
ical/metabolite in the blood of rats was consistently higher for nose-only exposures compared to body-only exposures for each of the four chemicals tested. The nose-
only / body-only blood concentrations ratios were 19 (toluene), 52 (dioxane), 61 (MEK), and 188 (isopropanol). The data from this study suggests that prediction of the body-only to nose-only blood ratios may be possible utilizing specific properties for a chemical (e.g., molecular weight, Kow). Testing with additional chemicals should clarify whether the blood concentration ratios between the two exposure modes and the resulting data could be used in determining dermal protection fac-
tors for individual chemicals that could be applied to existing health based occupa-
tional exposure criteria.

**494 FROM TOPICAL ANTIDOTE AGAINST SKIN IRRITANTS TO A NOVEL COUNTER-IRRITATING AND ANTI-INFLAMMATORY PEPTIDE.**

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The primary purpose of the present study was to investigate the mechanism of the counter-irritating activity of topical iodine against skin lesions induced by chemical and thermal stimuli. The hypothesis that iodine exerts its activity by inducing an endogenous anti-inflammatory factor was confirmed by exposing guinea pig skin to heat stimulus followed by topical iodine treatment and skin extraction. Injection of the extract into naïve guinea pigs reduced heat-induced irritation by 62%. The pro-
tective factor, identified as a new nonapeptide (histone H2A 36-44, H-Lys-Gly-
Asn-Tyr-Ala-Glu-Arg-Ileu-Ala-OH), caused reduction of 40% in irritation score in heat-exposed guinea pigs. The murine analog (H-Lys-Gly-His-Tyr-Ala-Glu-Arg-
Val-Gly-OH, termed IIM1) reduced sulfur mustard (SM)-induced ear swelling at a dose-dependent bell shape manner reaching peak activity at 1mg/kg. Cultured keratinocytes transfected with the peptide were more resistant towards SM than the control cells. The peptide suppressed oxidative burst in activated neutrophils in a concentration-dependent manner. In addition, the peptide reduced glucose oxidase-
and carrageenan-induced skin edema in mice. Apart from thermal and chemical-in-
duced skin irritation, this novel peptide might be of potential use in chronic dermal disorders, such as psoriasis and pemphigus, as well as non-dermal inflammatory diseases like multiple sclerosis, arthritis and colitis. (Supported by the US-Israel Binational Science Foundation 0378320)

**495 PREDICTING SKIN PERMEABILITY: INCORPORATION OF CHEMICAL MIXTURE EFFECTS INTO SIMPLE QUANTITATIVE STRUCTURE PERMEATION RELATIONSHIPS (QSPER).**

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Predicting the degree of dermal absorption of topically exposed chemicals is an im-
portant issue in both environmental and occupational risk assessment. Most risk as-
essment approaches and QSPeR models are based on data obtained from dosing chemicals neat or in simple aqueous vehicles, yet most exposures are to complex mixtures. We have previously demonstrated that a simple QSPeR model based on 12 penetrants in 24 mixtures could be constructed if the physical chemical proper-
ties of the mixture components and vehicle were also incorporated. The present study significantly expands on this analysis by increasing the number of penetrants studied using in vitro porcine skin diffusion cells to 16 for a total of 384 treatment combinations. In addition, we applied the model to 31 penetrants dosed in a total of 189 treatment combinations using an isolated perfused porcine skin model pre-
viously shown to be predictive of in vivo human absorption. These studies demon-
strated that mixture chemical descriptors including topical polar surface area/or ovality significantly improve prediction of dermal absorption using different base QSPeR models (e.g. Abraham 5-term or Potts and Guy 2-term models). These studies suggest that such information could be incorporated into dermal risk assess-
ment protocols to improve prediction of chemical absorption based on more realis-
tic exposure scenarios (Supported by NIOSH OH-07555)

**496 DEVELOPMENT OF QUANTITATIVE METHODS FOR ASSESSING SOLAR UV-INDUCED GENOTOXICITY ON RECONSTRUCTED SKIN: DNA DAMAGE, P53 STATUS, AND APOPTOSIS.**


Even if normal human keratinocytes in culture constitute a relevant and a validated model for photocogenticity studies, complementary information on 3D tissues in conditions closer to human skin is necessary. In this regard, industrial reconstructed skin models such as Episkin model are very convenient. The aim of this study was to provide quantitative methodology in order to precisely assess the genotoxic im-
pact of sunlight. Such methods should be faster and better adapted for screening purposes than classical immuno-histochemistry approaches. Here, a full thickness reconstructed skin model (including dermis with fibroblasts embedded in collagen) was exposed to simulated solar UV radiation similar to zenithal sunlight in terms of spectral power distribution. Biological endpoints related to genotoxicity were then
We conclude from these results that the newly developed melanocyte containing several active ingredients that (i.a.) were known to directly inhibit tyrosinase activity. Increasing melanin contents were observed after direct UV-radiation which addi- 

This characterisation included histological examinations to investigate the structural properties of the epidermis and immunohistochemical staining of the melanocytes by using anti-HMB45 antibodies. An important feature for testing effects on skin pigmentation is the feasibility of cultivating the models for at least ten days after substance application without significant loss of viability. Therefore, the models were cultured for different periods at the air liquid interphase followed by a standard MTT assay to determine their vitality. Finally, the melanin content for each test approach was measured. Increasing melanin contents were observed after direct UV-radiation which additionally lead to a visible tanning of the epidermis. This effect was strongly affected by several active ingredients that (i.a.) were known to directly inhibit tyrosinase activity. We conclude from these results that the newly developed melanocyte containing epidermal model is a useful tool for research and for characterisation of skin tanning or bleaching substances and for cognate applications which require model systems with melanocytes.

The development of new active compounds for skin bleaching and tanning are important issues for the cosmetic and pharmaceutical industry. However active ingredients have to be tested extensively for their efficacy and product safety. The aim of the present study was to create a reconstructed epidermis containing keratinocytes and melanocytes as a tool especially for efficacy studies. Based on the technology of the Epidermal Skin Test - EST1000 (CellSystems® Biotechnologie Vertrieb GmbH, Krefeld, NRW, Germany) several batches of skin models with different ratios of melanocytes and keratinocytes were produced and characterised. This characterisation included histological examinations to investigate the structural properties of the epidermis and immunohistochemical staining of the melanocytes by using anti-HMB45 antibodies. An important feature for testing effects on skin pigmentation is the feasibility of cultivating the models for at least ten days after substance application without significant loss of viability. Therefore, the models were cultured for different periods at the air liquid interphase followed by a standard MTT assay to determine their vitality. Finally, the melanin content for each test approach was measured. Increasing melanin contents were observed after direct UV-radiation which additionally lead to a visible tanning of the epidermis. This effect was strongly affected by several active ingredients that (i.a.) were known to directly inhibit tyrosinase activity. We conclude from these results that the newly developed melanocyte containing epidermal model is a useful tool for research and for characterisation of skin tanning or bleaching substances and for cognate applications which require model systems with melanocytes.

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The National Institutes of Health, National Institute of Allergies and Infectious Disease and Army Medical Research Institute of Chemical Defense (USAMRICD) with funding support through an Interagency Agreement (IAA) between the U.S. government and the European Union. This study was designed to develop new cosmetics in European Union. Common cytotoxicity of the skin is well known, and the use of these in vitro models to test efficacy and/or safety of new compounds and determine their mechanisms of action is necessary to acquire knowledge of their metabolic equipment and its performances. The characterization of metabolic capabilities for skin models developed by L’Oreal (EpicSkin™ and SkinEthic-RHE™) was initiated and compared with a normal human skin (NHS). It was managed by measuring the expression level of mRNA coding for phases 1 and 2 metabolizing enzymes and their catalytic activities. Main cytochrome P450 (CYP450) families involved in drug metabolism as well as N-acetyl (NAT) and Glutathione (GST) were already estimated. Estrofas (ES), glucuronidation (UGT) and sulfo (SULT) transferases were probed to get an overview of their catalytic activities. The microarray analysis were performed in both models and NHS using 4-methylumbelliferyl acetate (4-MUA), 4-methylumbelliferone (4-MU) and p-nitrophenol (PNP) as substrates, respectively. Apparent K_m and Km were determined using a single-well plate format (high-throughput ES). These values were the tissue and results showed a high ES activity, a much lower UGT activity and a very weak SULT activity detectable with phenol substrates while more strongly observed with steroids. To conclude, NHS and models are equipped well with hydrolytic and glucuronidation transferase activities and less for sulphanilic reactions. This study enriches the existing data on skin metabolism and indicates that reconstructed human skin models express the same functional metabolic equipment than a normal human skin and can be useful tools to design or select skin care molecules.

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Bromine is an industrial chemical that can cause severe cutaneous burns. Understanding the molecular mechanisms of tissue damage and wound healing is important for the selection and development of an effective treatment. This study investigated the effect of vapor bromine cutaneous exposure using a weaning swine burn model and microarray analysis. Ventral abdominal sites (N=3 per exposure group) were exposed to a mean calculated bromine vapor concentration of 0.51 g/L for 7 or 17 min. At 6 h, 48 h, and 7 days post-exposure, total RNA from skin samples were isolated, processed, and hybridized to Affymetrix GeneChip® Porcine Genome Arrays. Differences in gene expression were observed with respect to exposure duration and sampling time. Ingenuity Pathways Analysis (IPA) revealed four signaling pathways (HMOX1, ATF2, IL-8, TIMP1, ESR1, HSPAIL, and SELE) that were commonly shared among four significantly altered signaling pathways. Among these, the scripts encoding HMOX1 and ESR1 were identified using IPA as common potential therapeutic targets for Phase II/III clinical trial, or FDA-approved drugs. The model based on the 25 selected chemicals (R^2= 0.99) was statistically better then the model based on currently used subjective testing of 32 chemicals (R^2= 0.91) and Log Kp values (-3.99 to 1.58 cm/hr) covered a wider range than other documented training sets (-2.64 to -0.97). Our research demonstrated that this UCD method could significantly improve QSARs currently used in dermal risk assessments.

' L’Oréal, Aulnay Sous Bois, France and ‘SkinEthic Laboratories, Nice, France. Sponsor: G. Nohynek.

The reconstructed Human Epidermis tissues EpicSkin™ and SkinEthic-TM-RHE 42 bist test methods were validated by ECVM as stand alone replacement tests for the prediction of acute skin irritation discriminating irritant and non irritant substances (ESAC statements April 2007 and November 2008 respectively). Both methods were based on the quantification of tissue viability (MTT assay) and used similar principal strategies. Thus short treatment periods were used (15 minutes exposure for the Episkin™ test method and 42 minutes exposure for the RHE 42 bist test method) followed by a 42 hours post-treatment incubation period. Viability is quantified by a standard colorimetric method and during formazan extraction, any unspecific color remaining in the tissue or developed in situ may be extracted and consequently induce a possible false viability overestimation. The purpose of the study was to introduce specific controls allowing the use of the test methods for the irritation prediction of colored/coloring substances. After rinsing, colored/coloring substances could be retained in the epidermis, mainly in the stratum corneum. Unspecific colour was quantified by using treated tissue controls following the standard protocol course but incubated with medium instead of MTT. These specific controls enabled the quantification of the resulting non-specific color-related optical density (OD) and the correction of final true OD due to mitochondrial activity. Histological analysis can be conducted in order to document strong coloring substances (> 50% relative to negative controls). Using adapted controls, the applicability domain of both Episkin™ and SkinEthic-TM-RHE 42 bist test methods could therefore be considered compatible with colored/coloring test substances defined either irritant or non irritant.

' L’Oréal, Aulnay Sous Bois, France and ‘SkinEthic Laboratories, Nice, France. Sponsor: G. Nohynek.

The majority of earlier quantitative structure activity relationship (QSAR) were built from less diverse chemicals and with few descriptors, which were collinear with each other. The objective here was to develop a training set of chemicals representing a wider chemical space relevant to biological activity such as dermal permeability (Log Kp) and compare it with the currently used training set of chemicals by ADME boxes database was used for this study. The approach for diverse chemical selection was performed using Uniform Coverage Design (UCD) run by SpaceFill program and compared with a cluster analysis. Five sets of 25 chemicals were obtained from the design and evaluated based on gas chromatographic assay amenability and representation of structurally diverse group. A final set of 25 chemicals to be used for modeling dermal permeability was selected, based on aforementioned criteria and had molecular weights and Log Ko/w values ranging from 55 to 360 and -1.12 to 8.22, respectively. Graphical plot of the principal components demonstrated that currently used training sets of chemicals have narrow representation of parent dataset whereas the selected training set from UCD have appropriate representation in terms of chemical space. QSAR models were built from both training set of chemicals. The model based on the 25 selected chemicals (R^2= 0.99) was statistically better then the model based on currently used subjective training set of 32 chemicals (R^2= 0.91) and Log Kp values (-3.99 to 1.58 cm/hr) covered a wider range than other documented training sets (-2.64 to -0.97). Our research demonstrated that this UCD method could significantly improve QSARs currently used in dermal risk assessments.

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In line with the 7th deadlines, European Union bans the in vivo skin irritation assessment on ingredients for cosmetic purposes. Thus, the reconstructed human epidermis EpicSkin model test method was validated by ECVM (ESAC statement, 2007) as a stand alone test for the prediction of acute skin irritation. The validated test method is mainly based on tissue viability assessment (MTT) following a 15 minutes exposure and a 42 hours post-treatment incubation period. An implementation of the Global Harmonization System (GHS) in the European Union Classification (EU) has conducted to a new GHS-EU classification. The in vivo score cut -off value shifted from 2 to 2.3 for distinguishing non-irritant (no category) from irritant substances (category 2). Therefore, substances presenting in vivo scores comprised in this interval (GHS category 3) are considered as non irritants in the GHS-EU classification. As a result, this classification conducted to have more unbalanced working sets in terms of in vivo irritants versus non irritants (relating to

' L’Oréal, Aulnay Sous Bois, France and ‘SkinEthic Laboratories, Nice, France. Sponsor: G. Nohynek.
some extend the “real life” distribution of skin irritant potencies. Taking into ac-
count this GHS-EU update, global performances of the EpiSkin skin irritation test method were recalculated by merging working sets (including the ECVAM valida-
tion set) evaluated in our laboratory. Analyses were performed on an overall set of 103 chemicals composed of 1 quarter irritant and 3 quarters non irritant. Predictive capacities were defined by using the new GHS-EU classification and showed a sen-
sitivity increase together with a slight specificity decrease as compared to the EU classification. The overall performances of the EpiSkin test method were in accor-
dance with the criteria defined by ECVAM. However, these results evidenced the impact of in vivo classifications rules on in vitro methods performances.

506 DEER VELVET EXTRACT DECREASES THE GRADE AND METASTASIS OF AOM-INDUCED COLON CANCER IN THE MALE WISTAR RAT.

R. J. Rosengren 1, A. Frasier1, E. C. Stuart1, M. J. Scandlyn1, T. J. Somers-Edgar1, A. Alexander1 and S. R. Haines2.

Deer velvet (DV) extract has potential for use to promote healing of chronic wounds, as it increases angiogenesis. Therefore, the ability of DV to modulate the growth and invasiveness of colon carcinogenesis was investigated. Male Wistar rats were each given a subcutaneous injection of azoxymethane (AOM) at 15 mg/kg weekly for 2 weeks. One week following the final dose of AOM the rats were treated with either 1 g/kg of DV delivered in a cube of raspberry gelatin or a cube of un-
supplemented raspberry gelatin daily for 26 weeks. At necropsy, tumors were measured and the distance from the anus was recorded. Tissue samples were then and categorized according to the Astler-Coller system following histopathol-
gy. The results showed that there were no significant differences in most parame-
ters except for grade and metastasis. Once the DV treated rats received either 1 g/kg of DV delivered in a cube of raspberry gelatin or a cube of un-supplemented raspberry gelatin daily for 26 weeks. At necropsy, tumors were measured and the distance from the anus was recorded. Tissue samples were then and categorized according to the Astler-Coller system following histopathol-
gy. The results showed that there were no significant differences in most parame-
ters (i.e. body weight gain, multiplicity, tumor volume and incidence). For example, colon tumor multiplicity was 2.55 ± 0.32 for control and 2.41 ± 0.25 for control and DV treatments, respectively. The only statistically significant differences seen were associated with metastasis and tumor grade. Specifically, a higher proportion of the tumors in the DV treated rats were of a lower grade compared to the con-
trols, both when all tumor sites were considered (0.91 vs 0.66, p<0.0001), as well as those located only in the colon (0.95 vs 0.84, p=0.03). This study strongly demon-
strated that DV did not increase the incidence, multiplicity, metastasis or tumor volume of OAM-induced colon cancer. However, it did reduce metastasis and tumor grade. Therefore, the study provided no evidence that orally administered DV could promote colon carcinogenesis.

507 TRANSCRIPTIOINAL PROFILE OF DIUROM-INDUCED TOXICITY ON THE URINARY BLADDER OF MALE WISTAR RATS TO INFORM MODE OF ACTION.

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Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a substituted urea herbicide that induces rat urinary bladder urothelial tumors when provided at high dietary concentrations. There is little information regarding the cellular and molecular me-
terations that lead to toxicity and carcinogenicity of diuron in the rat urinary blad-
der. The transcriptional profiles of urinary bladder urothelial cells, eventual mu-
cosal histological alterations and dose-effects were evaluated in rats exposed to diuron. Six-week old male Wistar rats were exposed through diet to diuron at 0, 60, 1250, 2500 ppm levels for 20 weeks. Gene expression analyses of urothelial cells were conducted using Affymetrix GeneChip Rat Genome 230 2.0 whole-
genome Arrays (one chip per animal, three rats per dose group). One-way ANCOVA with a Benjamini Hochberg-FDR (false discovery rate) correction (5%) and Tukey-
Kramer post hoc test were used to test the obtained differentially expressed transcripts (DETs) (P ≤ 0.05) between each treatment group and controls. The number of DETs was 257, 291, 532 and 997 in the 60, 1250 and 2500 ppm treatment groups, respectively. Principal Components Analysis of the expression data sepa-
rated samples into two clusters; high doses (1250 and 2500 ppm) and low doses and control (0, 60 and 1250 ppm). The major categories of altered pathways after exposure to high doses included amino acid, lipid, phase I, and phase II metabo-
lism and oxidative stress response. Urothelial hyperplasia occurred in rats treated with 1250 and 2500 ppm diuron. These results suggest that extended exposure to

508 DERMAL CARCINOGENICITY EVALUATION OF ASPHALT (BITUMEN) FUME CONDENSATES.

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Canow/Phillips, Bartlesville, OK; 2BP Corporation, Napoleonville, LA; 3Marathon Petroleum, Findlay, OH; 4Asphalt Institute, Lexington, KY; 5ExxonMobil Biomedical Sciences, Inc., Annandale, NJ; 6Heritage Research Group, Indianapolis, IN and 7MPI Research, Mattawan, MI.

Asphalt (bitumen) fume condensates were collected from the headspace above paving (TPD) and Type III built up roofing (TRA) asphalt tanks and were evalu-
ated in 2-year dermal carcinogenicity assays in male C3H/HeNcCr mice. The fume condensate samples met pre-determined acceptance criteria that ensured their sim-
ilarly to field-generated fumes. A third sample (LRA) was generated from the same roofing asphalt using a NIOSH laboratory generation method. Roofing fume con-
densates were diluted in mineral oil and applied dermally twice per week (25 mg/application). The paving sample was applied 7 days/week at 7.14 mg/application in mineral oil to avoid dermal irritation noted in a 4-week pilot study. Exposure to TPD resulted in a single benign tumor (papilloma) at the end of the 2-
year study and only mild skin irritation was observed. Exposure to the LRA con-
densate resulted in statistically significant increases in squamous cell carcinomas (35) which confirmed the results of previous NIOSH studies on lab-generated fume condensates. The TRA condensate resulted in fewer carcinomas (8) with longer average latency (90 weeks vs 76 for LRA). Significant irritation was observed from TRA but delayed in onset until about week 52. It is concluded that LRA, which is not representative of field-generated fumes, was carcinogenic. TPD was not. While the TRA condensate was weakly carcinogenic, it’s low mutagenicity index and the marked skin irritation observed, suggests a non-genotoxic mecha-
nism might have been involved. To further investigate that possibility, the TRA sample is being evaluated in an initiation-promotion assay.

509 CONSENSUS DIAGNOSES AND MODE-OF-ACTION (MOA) FRAMEWORK FOR THE FORMATION OF GASTRIC NEUROENDOCRINE CELL TUMORS IN RATS TREATED WITH THE CHLOROANILIDE HERBICIDES ALACHLOR AND BUTACHLOR.


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A panel of pathologists was formed to evaluate histologically complex gastric tu-
mors and hyperplastic changes observed in the fundic region of rat stomachs in-
long-term carcinogenicity and gastric tumor mechanism studies with alachlor and butachlor. The intent of the review was to establish consistent diagnoses across all studies, to provide a Mode of Action (MOA) framework for the formation of the tumors, and to address why the tumors were histologically more complex than en-
terochromaffin-like (ECL) cell tumors observed after treatment with H2 receptor agonists (H2RAs) and proton pump inhibitors (PPIs). The panel examined H&E stained stomach sections and immunohistochemically stained tissues for ECL cells to determine the presence and relative proportion of ECL cells in the gastric tumors and hyperplasias. Phenotypic characteristics varied substantially among and within individual tumors. However, the panel concluded that all of the gastric tumors were of ECL cell origin. The MOA for the formation of the tumors includes the follow-
ing key events: profound mucosal atrophy, loss of parietal cells, hyperplasia of parietal and chief cells, hypergastrinemia, sustained increase in cell proliferation in the gastric fundic mu-
cosa, ECL cell hyperplasia, induction of ECL cell neoplasia, ECL cell paracrine and autocrine effects, and finally dedifferentiation of ECL cell tumors to fully developed tumors with variable phenotypic characteristics. Mucosal atrophy, loss of parietal cells, and compensatory proliferation are not observed after tumorigenic doses of H2RAs and PPIs. The conclusion of the Expert Panel was that both alachlor and butachlor induce histologically complex ECL cell gastric tumors via a novel, non-
genotoxic, threshold-mediated MOA that is initiated by profound mucosal atrophy and parietal cell loss.
510 REDOX CYCLING BY ENDOGENOUS 2- AND 4-HYDROXYESTROGEN CATECHOL METABOLITES IS ASSOCIATED WITH OXIDATIVE STRESS IN HUMAN BREAST EPITHELIAL CELL LINES.

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Estrogen exposure is a major risk factor in the etiology of breast cancer. Estrogens are known to alter transcription of estrogen-responsive genes by binding to nuclear receptors. Previous studies have demonstrated that equilin, a component of hormone replacement therapy, is converted to catechol metabolites; subsequent oxidation reactions generate redox-active ortho-quinones. One-electron reduction of these quinones to semiquinones by cytochrome P450 oxidoreductase (POR) and their subsequent reaction with molecular oxygen generates reactive oxygen intermediates (ROIs) that have been directly linked to the carcinogenic process. In the present studies we characterized redox cycling of four endogenous catechol estrogens: 4-hydroxyestrone, 2- and 4-hydroxyestradiol, and 2-hydroxyestradiol. We found that each catechol estrogen metabolite readily redox cycles and generates ROI in MCF-7 (estrogen receptor positive, tumorigenic), MDA-MB-231 (estrogen receptor negative, tumorigenic), and MCF-10A (normal immortalized) human breast epithelial cells, indicating that redox cycling occurs independently of the hormone receptor pathway. These cells were found to have similar capacities to produce hydrogen peroxide and hydroxyl radicals. Formation of ROI was associated with increased lipid peroxidation and DNA- and protein damage in intact cells. These data demonstrate that 1) endogenous catechol estrogens redox cycle, producing ROI; and 2) this process can induce cellular oxidative stress. Since breast epithelial cells readily metabolize estrogens to 2- and 4-hydroxy catechols, our data suggest that catechol estrogen redox cycling may contribute to the development of breast cancer. Supported in part by NIH grants ES05022, ES07148, AR055073, CA100994 and CA03798.

511 CIGARETTE-SMOKE-INDUCED CELLULAR TRANSFORMATION IN VITRO USING THE BHAS 42 CELL TRANSFORMATION ASSAY.


In vitro cell transformation assays detect transformed cells that have acquired the characteristics of malignant cells and thus mimic some stage of the in vivo multi-step carcinogenesis model. These assays have been proposed as surrogate models for predicting the non-genotoxic carcinogenic potential of chemicals. A short-term cell transformation assay using v-Ha-ras transfected Balb/c 3T3 cells (Bhas 42 cells), the Bhas assay, which has not been used with cigarette smoke before, is capable of detecting, initiating, and promoting activities of chemical carcinogens. As the particulate phase of cigarette smoke (total particulate matter, or TPM) is known to induce tumors in vivo in the mouse skin painting assay, we investigated the responsiveness of the Bhas assay to form morphologically transformed foci in vitro when repeatedly challenged with TPM from a standard reference cigarette (3R4F). DMSO-dissolved TPM induced a dose-dependent increase of type II foci in the Bhas cell transformation assay. A significant increase in focus formation was observed in the promotion assay at sub-toxic doses between 5 and 60 μg TPM/ml (16-fold). This novel in vitro assay using Bhas cells, which are regarded as initiated in the two-stage paradigm of carcinogenesis, is able to detect cell transformation induced by cigarette smoke condensate in a dose-dependent manner with a high dynamic range compared to the solvent control.

512 THE HYPOXI INDUCIBLE TRANSCRIPTION FACTOR HIF IS STABILIZED IN MICE TREATED WITH 2-BUTOXYETHANOL.

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2-Butoxyethanol (2-BE) causes hemolysis in mice and, following repeat administration, causes an increased incidence of hemangiosarcoma in the liver. A non-genotoxic mode of action (MOA) has been proposed in which hemolysis leads to hepatic iron accumulation, activation of local macrophages (Kupffer cells), oxidative damage and release of growth factors; responses that may stimulate clonal endothelial cell expansion and tumorigenesis. Recently, we have provided evidence that hypoxia may also play a key role in endothelial cell tumor development following treatment with 2-BE (Laifeild et al., 2009). Hypoxia activates the HIF (hypoxia inducible factor) transcription factor, which regulates a variety of signaling pathways that promote cellular proliferation and angiogenesis. Until now, we have only been able to indirectly demonstrate that HIF is activated in 2-BE treated mice by detecting changes in HIF-related gene expression. Here we use a genetically modified mouse engineered to emit light from tissues where HIF is stabilized to provide direct evidence that 2-BE produces HIF stabilization in vivo. ROX26 ODD-Luc +/- mice (Safaran, et al. 2006) were treated with a single dose of 900 mg/kg 2-BE and imaged using an IVIS bioluminescent imager. Mice were treated with a prolyl hydroxylase 2 (PHD2) inhibitor as a positive control. We detected a significant increase in light emitted from the abdominal region of the mice ths following 2-BE administration consistent with decreases in RBC parameters at this time. This local HIF stabilization may contribute to a growth factor environment, coupled with the macrophage effects previously reported, that stimulates endothelial cell proliferation and hemangiosarcoma.

513 MOUSE ENDOTHELIAL CELL SURVIVAL AND PROLIFERATION REQUIRE UPREGULATED ANGIOPOIETIN-2 UNDER HYPOXIA.

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Hypoxia has been identified as part of a unified Mode of Action for non-genotoxic hemangiosarcoma formation in mice (Cohen et al. 2009). Hypoxia activates the HIF (hypoxia inducible transcription factor, or HIF) by inducing pathways that promote cellular proliferation and angiogenesis. One of the genes upregulated in endothelial cells under hypoxia is angiopoietin-2 (ANG-2). Ang-2 is a ligand for the endothelial cell Tie-2 receptor, is important for vasculogenesis and remodeling, and enhances cell survival through PI3K/AKT signaling under conditions that promote apoptosis (Kim et al., 2000). We hypothesized that ANG-2 may promote endothelial cell survival and proliferation under hypoxic conditions, and thereby may play a key role in mouse hemangiosarcoma formation. Mouse endothelial cells (3B11 and SVEC4-11) were incubated under 1% O2 or transduced with shRNAs targeting the prolyl hydroxylase-2 (PHD-2) enzyme in order to stabilize HIF. Both treatments increased endothelial cell proliferation and upregulated ANG-2. PHD-2 knockdown also increased phosphorylated AKT. An shRNA targeting ANG-2 showed that ANG-2 knockdown blocked endothelial cell proliferation under hypoxic, but not normoxic, conditions. We conclude that ANG-2 is required for survival and proliferation of mouse endothelial cells in hypoxic environments, and it may be a key component of mouse hemangiosarcoma formation.

514 WEIGHT-OF-EVIDENCE ANALYSIS OF HYDROQUINONE AND LEUKEMIA.

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Hydroquinone (HQ) is a naturally occurring chemical, found in certain foods and used in skin bleaching products and in film processing. It is also one of the metabolites of benzene, a known leukemogenic agent. Because (a) HQ has been found in bone marrow of animals after exposure to benzene, (b) HQ has been shown capable of bone marrow effects in genotoxicity assays, and (c) benzene leukemogenicity is believed to be caused by its metabolites (including HQ), we reviewed the available scientific evidence to examine whether the characteristics of benzene leukemogenicity are relevant to the potential for HQ exposure alone to cause leukemia in humans. Whereas benzene is known to be leukemogenic in sufficiently exposed workers, occupational studies involving HQ exposure do not support an association between HQ and leukemia or any other type of cancer. With the exception of renal adenomas in HQ-exposed male rats, there are no consistent tumor findings in animals following HQ exposure but not after plausible human exposures to HQ. Only exposures to HQ at high concentrations and/or administered parenterally (neither are relevant to human exposures) will overwhelm the detoxification pathways in the liver and allow HQ to enter the bloodstream and reach the bone marrow. It is almost exclusively under these conditions that positive cytogenetic toxicity results of...
HQQ in animals are derived. In contrast, there is convincing evidence of benzene cytogenetic toxicity from human exposures. We conclude that, despite the potential role of HQ in benzene leukomogenicity, the weight of scientific evidence does not support that HQ alone causes leukemia in humans.

515 ASCORBIC ACID INHIBITS COLON CANCER CELL GROWTH BY INDUCING ROS-DEPENDENT DOWNREGULATION OF SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS.

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Ascorbic acid is an important nutrient commonly considered as an anti-oxidant. However in previous studies it was also shown that ascorbate at pharmacological concentrations (mM) acts as prooxidant, generating hydrogen peroxide dependent cytotoxicity in a variety of cancer cells in vitro without adversely effecting normal cells. In this study, treatment of SW480 and RKO colon cancer cells with 2-5 mM ascorbic acid decreased cell proliferation and the IC50 value for growth inhibition was approximately 5 mM in both cell lines. In this same concentration range ascorbic acid also induced apoptosis, decreased expression of survivin, vascular endothelial growth factor (VEGF) and VEGF receptor1 (VEGFR1). Previous studies show that expression of survivin, VEGF and VEGFR1 in cancer cell lines is dependent on specificity protein (Sp) transcription factors Sp1, Sp3 & Sp4 and therefore we investigated the effects of ascorbic acid on these transcription factors in SW480 and RKO colon cancer cells. At concentrations as low as 3 mM, ascorbic acid decreased expression of Sp1, Sp3 and Sp4 proteins in both cell lines. These results suggest that one of the underlying mechanisms of action of ascorbic acid as a prooxidant anti-cancer agent in colon cancer cell lines is the targeted degradation of Sp transcription factors, which results in activation of growth inhibitory, antiangiogenic and proapoptotic pathways. Moreover, mechanistic studies suggest that ascorbate induced effects on Sp proteins were dependent on reactive oxygen species (ROS) and cotreatment of colon cancer cells with ascorbate plus antioxidant such as glutathione produced a synergistic effect on the anti-proliferative and proapoptotic activity of ascorbic acid. Moreover, increased expression of survivin, VEGF and VEGFR1 was observed in normal colon epithelial cells suggesting that ascorbic acid may also be a prooxidant anti-cancer agent in normal colon epithelial cells.

516 EFFECTS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARγ) AGONISTS ON ENDOTHELIAL CELLS: DIFFERENCES BETWEEN SARCOMAGENIC TROGLITAZONE AND NON-SARCOMAGENIC PIOPILGIOZTANE.

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A common tumor finding in bioassays of nongenotoxic PPARγ agonists aimed at drug discovery is the development of tumors at the site of injection that were not present in vehicle controls. This finding that nongenotoxic PPARγ agonists are sarcomagenic. This finding is important as the use of PPARγ agonists aimed at drug discovery is expected to increase. Consequently, new criteria for determining the potential of PPARγ agonistinduced tumors are needed. We previously showed that troglitazone (TG) increased endothelial cell (EC) proliferation in mice at sarcomagenic doses, and TG directly increased DNA synthesis and inhibited apoptosis in mouse (MFP MVEC) but not in human (HMEC1) microvascular ECs. We postulated that TG increased EC proliferation through these direct effects on mouse ECs in part leading to HS formation. To test our hypothesis, the effects of the non-sarcomagenic PPARγ agonist pioglitazone (PIO) were examined. EC proliferation in liver was not changed with TG, PIO or control treatment in either rat or mouse. In contrast, PIO treatment caused an increase in HMEC1 and MVEC EC proliferation in vitro. Interestingly, HMEC1 proliferation was not as effective as MVEC proliferation. The increase in EC proliferation in MVEC was concentration dependent. TG increased HMEC1 EC proliferation in a concentration-dependent manner in HMEC1 and MFP MVEC on day 3. MFP MVEC were more resistant to the cytototoxic effects of PIO compared to HMEC1. We postulated that TG increased EC proliferation through these direct effects on mouse ECs in part leading to HS formation. To test our hypothesis, the effects of the non-sarcomagenic PPARγ agonist pioglitazone (PIO) were examined. EC proliferation in liver was not changed with TG, PIO or control treatment in either rat or mouse. In contrast, PIO treatment caused an increase in HMEC1 and MVEC EC proliferation in vitro. Interestingly, HMEC1 proliferation was not as effective as MVEC proliferation. The increase in EC proliferation in MVEC was concentration dependent. TG increased HMEC1 EC proliferation in a concentration-dependent manner in HMEC1 and MFP MVEC on day 3. MFP MVEC were more resistant to the cytototoxic effects of PIO compared to HMEC1. We postulated that TG increased EC proliferation through these direct effects on mouse ECs in part leading to HS formation.

517 GLOBAL GENE EXPRESSION ANALYSIS REVEALS DIFFERENCES IN CELLULAR RESPONSES TO HYDROXYL- AND SUPEROXIDE ANION RADICAL-INDUCED OXIDATIVE STRESS IN CACO-2 CELLS.


Reactive oxygen species-induced oxidative stress in the colon is involved in inflammatory bowel diseases and suggested to be associated with colorectal cancer risk. However, our insight in molecular responses to different oxygen radicals is still fragmentary. Therefore we studied global gene expression by an extensive time serie (0.08, 0.25, 0.5, 1, 2, 4, 16, or 24 hours) analyses in human colon cancer (Caco-2) cells after exposure to H2O2 or the superoxide anion donor menadione. Differences in gene expression were investigated by hybridisation on two-color micro arrays against non-exposed time-matched control cells. Correlations with related phenotypic markers (oxidative DNA damage, cell cycle arrest) were investigated. Gene expression analysis resulted in 1404 differentially expressed genes upon H2O2 challenge and 979 genes after menadione treatment. Further analysis of gene expression data revealed how these oxidant-responses can be discriminated. Time-dependent co-regulated genes immediately showed a pulse-like response to H2O2 while the menadione-induced expression is not restored over 24 hours. Pathway analyses demonstrated that both H2O2 and menadione strongly modulate cell cycle-related pathways. Correlating gene expression changes with changes in 8-oxoG and cell cycle phases revealed relevant genes and pathways, e.g. CDKN1C that is clearly involved in the regulation of cell cycle progression. The results of this study offer a novel and detailed insight in the similarities and differences of the time-dependent stress responses induced by the oxidants H2O2 and menadione and show that these changes support that HQ alone causes leukemia in humans.

518 CAMP-DEPENDENT PATHWAY(S) DIRECTS THE RAP-GTP/B-RAF MAPK-MEDIATED CYTOSOLIC MISLOCALIZATION OF P27/KIP-CYCLIN D1 IN RE NAL CANCER.

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The loss of tuberin, the tuberous sclerosis-2 (Tsc-2) gene product, is associated with cytoplasmic mislocalization of p27 in uterine leiomyomas derived from Eker rats (Tsc-2/EK+)/, and has been recently observed in human renal cell carcinoma tissue. Signaling associated with cytoplasmic mislocalization of p27 is relatively unknown, and has not been studied in renal tumor development. Renal tumors, null for tuberin, develop from 4.3-tris-glutathion-S-ylhydroquinone (TGHQ) treated Tsc-2/EK+ rats, display elevated nuclear and cytotoxic p27, and a parallel increase in cytosolic cyclin D1 levels. Similar changes were observed in TGHQ transformed renal epithelial cells derived from Tsc-2/EK+ rats (QTRRE cells), which, in addition to the cytoplasmic mislocalization of p27 and cyclin D1, also exhibit high ERK, B-Raf and Raf-1 kinase activity. Renal tumor xenographs, derived from subcutaneous injection of QTRRE cells into nude mice, also displayed increased cytosolic mislocalization of both p27 and cyclin D1. Dibutyryl cAMP and/or phosphodiesterase inhibitors (IPIs; pentoxifylline or theophylline) cause an increase in Rap1 activation, B-Raf kinase activity, and cytosolic p27/cyclin D1 protein levels in QTRRE cells. Additionally, treatment of QTRRE cells with both PIs revealed concomitant decreases in p27 and cyclin D1. However, our insight in molecular responses to different oxygen radicals is still fragmentary. Therefore, the loss of tuberin and engagement of the cAMP pathway may independently direct p27/cyclin D1 stabilization during renal tumor formation. (GM039338, P30ES006694, T32ES007091).
prostate cancer cells. All 8 BEL derivatives induced concentration- and time-de-
pendent cytotoxicity as determined by MTT staining and cellular morphology. The IC₅₀ of BEL was 9 μM in LNCaP cells and 14 μM in PC-3 cells after 72 hr. The bromo-base of BEL (BB), which contained a lactone ring without naphtha-
lene attached, had IC₅₀ values similar to BEL. Replacement of the bromo-leaving group with iodide did not alter the IC₅₀ compared to BB, suggesting that the leaving group of BEL is not a determinant of toxicity. Conversely, a monophenyl-bro-
moenol lactone (MP-BEL) had IC₅₀ values of 4 and 6 μM in LNCaP and PC-3 cells, while an S-enantiomer monophenyl analog of BEL containing a propargyl group attached to nitrogen in the 4 position of the lactone (S-TEL-III-19) had IC₅₀ values of 3 and 5 μM. The addition of a carbon bond between the lactone and the phenyl rings of TEL-III-19 increased IC₅₀ values 3-fold compared to BEL, suggest-
ing that this site is a key determinant of toxicity. Neither the replacement of the bromo-leaving group of TEL-III-19 with iodide (TEL-III-35A), nor the addition of a propargyl group at the 4 position of a bromo-base (TEL-III-37A), increased toxicity, compared to TEL-III-19. In contrast, the R-enantiomer of TEL-III-19 (R-
TEL-III-44A) had the lowest IC₅₀ in both cell lines, suggesting that stereochemistry mediates the anti-cancer activity of BEL derivatives. Assessment of iPLA₂ activity in rat kidney cytosol demonstrated that BEL, MP-BEL and S-TEL-III-19 all inhib-
ited activity, while none of the other compounds did. Collectively, these data demonstrate that derivatives of BEL are toxic to prostate cancer cells, and identify novel structure-activity relationships that dictate both their toxicity and ability to inhibit iPLA₂.

520 EVIDENCE FOR FORMALDEHYDE-INDUCED LEUKEMIA AND LYMPHOMA IN LONG-TERM RODENT BIOASSAYS.


Epidemiologic studies indicate an association between lymphohematopoietic (LHP) malignancies and formaldehyde exposures for various occupations. Specific diseases and disease groupings reported include: all LHP, all leukemia, myeloid leukemia (in some cases chronic myeloid leukemia), Hodgkin's Disease and Multiple Myeloma. These findings have resulted in considerable debate regarding the biological plausibility of formaldehyde-induced LHP cancer. Although animal inhalation studies have not reported formaldehyde-induced leukemia and leu-
phoma, study designs - including lack of complete histopathological examinations, study size and study duration - present limitations in the published literature. We identified one study where detailed organ histopathology data were available for analysis (Battelle Laboratories, 1999). Fischer 344 rats and B6C3F1 were exposed to formaldehyde for up to 24 months (both sexes: 15 ppm or 80 ppm, 8 hrs/day, 5 days/week for 24 mos.). All data were reabstracted and tumor incidence for treated animals was appropriately adjusted for early deaths and time to tumor observation. Our analysis revealed evidence for increased lymphoma in female B6C3F1 mice (28% versus 18%) and increased leukemia in female F344 rats (24% versus 15%). No increase in leukemia incidence was found in treated male rats. Both rat leukemia and mouse lymphoma increased in lifetime tumors, and this study may have had an advantage over other published studies with animals examined up to 6 months after the 24 month exposure period. Additionally, analysis of tumor bear-
ing animals, versus specific sites (e.g. submandibular versus spleenic lymphomas) increased the sensitivity of the bioassay. These results are consistent with increased formaldehyde-induced lymphohematopoietic malignancies reported for a lifetime drinking water study in rats (Solfritti et al., 2002). Overall, these two studies pro-
vide support for the plausibility of formaldehyde-induced LHP cancer. Disclaimer: This abstract does not necessarily reflect EPA policy.

521 EVALUATION OF THE MODE OF ACTION (MOA) FOR LIVER AND THYROID TUMORS IN MALE F344/DCRRL RATS WITH BENFLURALIN.

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Benfluralin (N-Butyl-N-ethyl-2,6-dinitro-4-(trifluoromethyl)benzenamine) is a pre-emergence dinitroaniline herbicide used to control grasses and other weed species. In a previously conducted 2-year rat carcinogenicity study, liver and thyroid tumors were observed in male F344 male rats exposed to 5000 ppm benfluralin. The purpose of this study was to examine a possible mode of action (MOA) for the benfluralin-induced liver and thyroid tumors by exposing male F344 rats to a car-
cinogenic dose of 5000 ppm benfluralin in the diet for 7 or 14 days. Absolute and relative liver weights were significantly elevated at 7 and 14 days and correlated with microscopic observation of centrilobular/midzonal hypertrophy. Absolute and relative thyroid weights were also statistically elevated and correlated with micro-
scopic changes showing very slight follicular cell hypertrophy. Targeted gene expres-
sion of Cyp1a1 and Cyp1b1 (constitutive androstane receptor [CAR]-associated genes) were significantly elevated 47- and 11.45-fold, respectively at 7 days and 344- and 12.09-fold, respectively at 14 days. Cyp1a3, Ugt1a6, and Ugt2b1 were significantly elevated at both 7 and 14 days: Cyp4a10, a PPARα-associated gene, was not altered in this study. At 7 days, T3 and T4 thyroid hormone levels were sig-
nificantly decreased, while only T4 remained significantly decreased at 14 days. Liver EROD, PROD, and UGT-related enzyme activity were significantly elevated at both 7 and 14 days. Hepatocellular proliferation (via BclU incorporation) was significantly elevated in the perportal region at 7 days and in all regions (centrilobu-
lar, midzonal, and periportal) at 14 days. Thyroid proliferation was significantly elevated only at 7 days. The responses observed in this study support CAR- and UGT-mediated MOAs for the observed benfluralin-induced liver and thyroid tu-
mors, respectively, both of which have little to no relevance to humans.

522 ARYL HYDROCARBON RECEPTOR (AHR) ACTIVATION DELAYS DMBA-INDUCED MAMMARY TUMOR FORMATION WITHOUT AFFECTING TUMOR INITIATION.

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Breast cancer is among the leading killers of women and there is a need for develop-
ning novel therapies and preventative measures. One proposed treatment strategy is the use of selective AhR modulators. TCDD (2,3,7,8-tetrachlorodibenzo-p-
dioxin), the best-characterized AhR ligand, has been shown to protect against mammary tumors in some breast cancer models. Previously we have shown that adult mice pre-treated with TCDD demonstrated delayed DMBA-induced mam-
mary tumor formation. The current studies were performed to elucidate the mech-
anism of the delay by examining the impact of prior AhR activation on the initia-
tion stage of carcinogenesis. Specifically we examined whether mice treated with TCDD four weeks prior had (i) persistent induction of metabolic enzymes that biotransform DMBA, (ii) decreased numbers of DMBA-DNA adducts, or (iii) di-
iminished proliferation of mammary epithelial cells. The results of these studies showed the levels of Cyp1a1 and Cyp1b1 in the mammary gland and liver were not substantially elevated four weeks later (the time of DMBA administration in the tumor study). There was also no effect of prior TCDD treatment on the other markers of initiation examined. These results are consistent with decreased expression of these receptors is hypothesized to slow promotion. However TCDD had little to no effect. Finally, we measured the expression of Cxcl12 and Cxcr4, as a link has been proposed between diminished expression of these fac-
tors and decreased breast cancer metastasis. Both molecules were significantly in-
creased in glands immediately following treatment with TCDD; however, this change did not persist. Taken together, these results suggest that prior AhR activa-
tion does not reduce carcinogen-induced tumor initiation, and current studies are examining additional effects on later stages of carcinogenesis.

523 DOSE-DEPENDENT INDUCTION OF HEPATIC PRENEOPLASTIC LESIONS BY DIETHYLNITROSAMINE IN C57BL/6 MICE.

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The C57BL/6 strain is used in bioassays and also as the background strain for many transgenic and knockout mouse models. These models have been used extensively to study the carcinogenesis process, and in particular, hepatocarcinogenesis. The C57BL/6 mouse strain is particularly low in susceptibility to both chemically-in-
duced and spontaneous liver tumor development. It is well established that hepato-
carcinogenesis in rodents is a multi-step process. Many hepatocarcinogens function at the promotion stage of the cancer process. The utilization of different mouse strains in mechanistic studies has proven beneficial to understanding the action of these nongenotoxic carcinogens. In the current study, we investigated the induc-
tion of preneoplastic and neoplastic lesions in the C57BL6 strain using diethylni-
trosa mine (DEN) treatment in a dose response manner. C57BL6 mice were treated weekly with 25, 50 or 75 mg/kg of DEN for 4 or 8 weeks by i.p. injection. Mice were examined for neoplastic and preneoplastic lesions at 26 weeks post treat-
ment. Using stereological techniques, the number, type, relative area and size of foci were determined. Additionally, a histopathological analysis of the liver revealed...
hepatic adenomas, a cholangioma, and a hemangioma following treatment. From these results, a suitable DEN range for inducing lesions in C57BL/6 mice was concluded to be 300-400 mg/kg DEN using either the 75 mg/kg for 4 weeks or 50 mg/kg for 8 weeks treatment protocol. These results will be useful for studies investigating hepatic tumor promotion in transgenic or knockout mice that use the C57BL/6 background strain (Supported by NIH CA100908 (JEK)).

Activation of Kupffer cells (KC) results in the release of inflammatory mediators, growth factors, and ROS. Previous studies suggest that KC exhibit growth permissive effects on hepatic cancer development. The present study further explores the role of KCs in hepatic tumor promotion by examining their role on bi(2-ethylhexyl)phthalate (DEHP)- and phenobarbital (PB)-induced hepatocellular proliferation and growth in naive and preneoplastic lesion-containing C57BL/6 mice. In the naive mouse study, 6-8 wk old male C57BL/6 mice were treated with PB (500 ppm in drinking water) and DEHP (500 ppm in diet) for 7 and 14 days. Lipopolysaccharide (LPS; 1 mg/kg, ip, 2x/wk) was used as a positive control. To induce preneoplastic lesions, male 21 day old C57BL/6 mice were given DEN (50 mg/kg, ip, 1x/wk for 4 wk). After focal lesions were apparent, mice were treated with DEHP or PB for 28 days. In both studies, KC were depleted with clodronate encapsulated liposomes (10 ml/kg, iv, 2x/wk). In naive animals, PB and DEHP increased hepatocellular DNA synthesis (6-fold and 2-fold over control, respectively) while KC depletion significantly reduced DNA synthesis. In mice with preneoplastic lesions, PB and DEHP produced 2-fold increases in focal lesion volume, and increased the rate of DNA synthesis within lesions, while depletion of KCs reduced focal lesion volume and DNA synthesis to that of control. In the surrounding non-lesion tissue, PB and DEHP marginally increased DNA synthesis relative to the control group which was also decreased by KC depletion. To address whether KC-derived ROS participate in hepatic tumor promotion, PB-induced DNA synthesis was evaluated in NADPH oxidase knockout mice (gp91-/-). DNA synthesis in PB-treated mouse liver was similar in gp91-/- mice compared to wild-type, suggesting that Kupffer cells derived ROS do not participate in PB-induced tumor promotion. Collectively, these results provide further support for KCs in hepatic tumor promotion (Supported by NIH CA100908).

**525** ALTERED DISTRIBUTIONS OF CALCIUM(2+) IONS IN NICKEL AND MCA-TRANSFORMED 10T1/2 MOUSE EMBRYO CELL LINES.

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C3H/10T1/2 mouse embryo fibroblast cells were treated with the carcinogens NiO, NIS, and MCA. Transformed foci were induced, ring-cloned, expanded into transformed cell lines, and characterized. Non-transformed 10T1/2 cells expressed mRNA of the vitamin D receptor interacting protein #80 (DRIP-80) in mRNA differential display experiments; NiO/NIS-transformed cell lines did not. DRIP-80 protein is a subunit of Mediator complex, which regulates vitamin D responsive genes involved in intracellular Ca+2 ion distribution. We hypothesized that Kupffer cell-derived ROS do not participate in PB-induced tumor promotion. To test this hypothesis, non-transformed/transformed 10T1/2 cell lines were stained with Ca+2 ion-binding fluorophores, Fluo 3-AM, and intracellular distribution of Ca+2 ions were visualized by confocal microscopy. In non-transformed 10T1/2 cells, some cells had high concentrations of Ca+2 ions in the nucleus and lesser amounts in the cytoplasm (State 1). Other cells had few Ca+2 ions in the nucleus; most of the Ca+2 ions were in the cytoplasm (State 2). These results suggest 1) 10T1/2 cells cycle between State 1 and State 2, and 2) silencing DRIP-80 gene in transformed 10T1/2 cell lines disrupts transport of Ca+2 ions between nucleus and cytoplasm and contributes to induction/maintenance of transformed phenotypes in NiO-2/MAA-transformed 10T1/2 cell lines. Supported by Grant ES03341 from NIEHS/NIH, M. S. program in Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

**526** EFFECTS OF ANTIOXIDANTS ON ACRYLONITRILE-INDUCED OXIDATIVE STRESS IN FEMALE F344 RATS.

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Acrylonitrile induces oxidative stress and damage in rat brain and is believed to be involved in the development of brain tumors seen in rats upon chronic exposure. The present study examined the effects of dietary antioxidant supplementation on acrylonitrile-induced oxidative stress and oxidative damage in rats in vivo. To assess this, female F344 rats were provided diets containing vitamin E (0.05%), green tea polyphenols (GTP; 0.4%), N-acetyl cysteine (NAC; 0.8%), and selenium (0.1 mg/kg), and taurine (10 g/kg) for 7 days, and then co-administered 0 and 100 ppm acrylonitrile in drinking water for 28 days. Significant increases in oxidative DNA adduct formation in brain (-2-fold over control), as evidenced by elevated 8-OhdG levels, were seen in acrylonitrile-exposed rats. Supplementation with vitamin E, GTP, and NAC reduced acrylonitrile-induced oxidative DNA damage in the brain while no protective effects were observed with the selenium or taurine supplementation. In addition, acrylonitrile increased oxidative DNA damage in white blood cells (-2-fold over control) measured by the fpg-modified alkaline Comet assay, which was reduced by supplementation of Vitamin E, GTP, NAC, selenium, and taurine. These results suggest that acrylonitrile mediates damage through mechanisms that involve suppression of inflammatory responses, inhibition of cell proliferation and stimulation of apoptosis. (Supported in part by the Acrylonitrile Group Inc.)
metallic nickel nanoparticles. Activator protein-1 (AP-1) and nuclear factor-kB (NF-kB) have been shown to play pivotal roles in tumor initiation, promotion, and progression. The present study examines effects of metallic nickel particles on tumor promoter or suppressor genes and its signal transduction pathways in JB6 cells. Our results demonstrated that metallic nickel nanoparticles activated AP-1 and NF-kB more efficiently than nickel fine particles as investigated using luciferase assay, western blot, as well as immunocytochemistry staining. Further studies indicated that, compared to fine particles, nickel nanoparticles induced a higher level protein expression of c-myc and R-Ras in a time-dependent manner. Furthermore, nickel nanoparticles caused a greater decrease of p53 transcription activity than fine particles as demonstrated by luciferase assay. These findings suggest that nickel nanoparticles may exhibit higher carcinogenic potential than nickel fine particles. The results obtained from this study will be of benefit for elucidating the pathogenic and carcinogenic potential of metallic nickel nano- and fine particles. In addition, the results may be useful as a reference when comparing the carcinogenicity of different nickel compounds.

**529 OXIDATIVE STRESS AND XRCC1 ARG399GLN AND XPC LYS939GLN POLYMORPHISMS IN A TURKISH POPULATION WITH GASTRIC CANCER.**

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According to the limited knowledge about molecular and genetic mechanisms of gastric cancer, it is still the most common mortality and morbidity cause, worldwide. Accumulation of constantly generated reactive species during cellular metabolism and extra cellular processes, may contribute to the process of carcinogenesis by oxidative DNA damage, while the synthesis of nitric oxide (NO) might interfere with DNA repair genes. X-ray repair complementing defective repair in Chinese hamster cell (XRC1) is one of the prominent base-excision repair enzymes, however the bulky adducts are excised by nucleotide excision repair enzymes, including xeroderma pigmentosum C (XPC). Association between the polymorphisms of DNA repair genes XRCC1 Arg399Gln, XPC Lys939Gln and the extent of oxidative stress were assessed in eligible 93 gastric cancer patients. One hundred and eight matched controls were included to the study. Genotypes were determined by PCR-RFLP using DNA extracted from peripheral blood cells. Albumin which is the major and predominant circulating antioxidant in serum, was measured by auto-analyzer. Serum NO was determined as nitrite levels by spectrophotometric method. Gastric cancer risk was showed to be 2.6 times higher in patients carrying XRCC1 homozygote Gln alleles at codon 399 (OR=2.614, 95% confidence interval: 1.036-6.597). Homozygote variant allele Gln of XPC at codon 939 was found to be not associated with increased risk of gastric cancer (0.575 (0.249-1.326)), despite of the increased oxidative stress that is indicated by the decreased levels of serum albumin (p<0.05). Significantly decreased serum NO concentrations (p<0.05) might be related with the insufficient tumoricidal activity and poor prognosis of the gastric cancer, and decreased nitrite levels of variant allele carriers of XPC at codon 939 might indicate an alteration in the repair of DNA of gastric cancer patients, as well.

**530 EFFECT OF SULINDAC DERIVATIVES ON SPECIFICITY PROTEIN TRANSCRIPTION FACTORS IN COLON CANCER CELLS.**

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Specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 are overexpressed in various types of human cancer cells in tumors. Their C-H3-type zinc fingers bind GC-rich promoter elements of genes that are critical for cancer cell survival, angiogenesis and proliferation. Since Sp transcription factors play a crucial role in maintaining the cancer phenotype, we have focused on investigation of drugs that target Sp protein downregulation. Sulindac sulfide, a metabolite of the non-steroidal anti-inflammatory drug (NSAID) sulindac, exhibited potent antiangiogenic activity in SW480 and RKO colon cancer cell lines, with an IC50 value (24 hr) of 50 μM in both cell lines. In contrast, sulindac (the sulfone) and sulindac sulfone were much less active, with growth inhibitory IC50 value at least seven times higher than observed for sulindac sulfide. Treatment with sulindac sulfide also induced downregulation of Sp1, Sp3, Sp4 proteins in SW480 and RKO cells. Sulindac sulfide also decreased expression of several Sp-regulated genes including survivin, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and cyclin D1. The mechanism of sulindac sulfide-induced anticancer activity was also investigated and antioxidants such as glutathione inhibited sulindac sulfide-dependent growth inhibition and downregulation of Sp1, Sp3, Sp4 and Sp-dependent gene products. Sulindac sulfide also induced reactive oxygen species (ROS) which was a critical element for the effect of this compound on growth inhibition and Sp downregulation in colon cancer cells. The results suggest that downregulation of Sp proteins contribute to the chemotherapeutic effects of sulindac and its metabolites observed in clinical studies with this NSAID.

**531 ARSENIC TROXIDE DOWNREGULATION OF SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS IN BLADDER CANCER CELLS IS DEPENDENT ON REACTIVE OXYGEN SPECIES (ROS).**

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Arsenic trioxide exhibits antiproliferative, antiangiogenic and proapoptotic activity in cancer cells, and many genes associated with these responses are regulated by specificity protein (Sp) transcription factors. Treatment of cells derived from urologic (bladder and prostate) and gastrointestinal (pancreas and colon) tumors with arsenic trioxide demonstrated that these cells exhibited differential responsiveness to the antiproliferative effects of this agent and this paralleled their differential expression of Sp1, Sp3 and Sp4 proteins in the same cell lines. Using arsenic trioxide responsive KU7 and non-responsive 253JB-V bladder cancer cells as models, we show that in KU7 cells, ≤ 5 μM arsenic trioxide decreased Sp1, Sp3 and Sp4 and several Sp-dependent genes and responses including cyclin D1, epidermal growth factor receptor, bcl-2, survivin and vascular endothelial growth factor, whereas at concentrations up to 15 μM, minimal effects were observed in 253JB-V cells. Arsenic trioxide also inhibited tumor growth in athymic mice bearing KU7 cells as xenografts, and expression of Sp1, Sp3 and Sp4 was significantly decreased. Inhibitors of oxidative stress such as glutathione or dihydrothreitol protected KU7 cells from arsenic trioxide-induced antiproliferative activity and Sp repression, whereas glutathione depletion sensitized 253JB-V cells to arsenic trioxide. Mechanistic studies suggested that arsenic trioxide-dependent downregulation of Sp and Sp-dependent genes was due to decreased mitochondrial membrane potential and induction of reactive oxygen species, and the role of peroxides in mediating these responses was confirmed using hydrogen peroxide.

**532 SYNTHETIC OLEANOLIC ACID-DERIVED TRITERPENOIDs INHIBIT BLADDER CANCER CELL GROWTH AND SURVIVAL AND DOWNREGULATE SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS.**

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Methyl 2-cyano-3,11-dioxo-18β-eleman-1,12-dien-30-oate (CDDO-Me) is a synthetic triterpenoid derived from glycyrrhetinic acid which inhibits proliferation of KU7 and 253JB-V bladder cancer cells with inhibitory IC50 values < 5 μM. CDDO-Me-dependent growth inhibition is accompanied by caspase-dependent PARP cleavage and downregulation of survival (survivin and bcl-2) and angiogenic (vascular endothelial growth factor (VEGF) and its receptor (VEGFR1)) genes. Arsenic trioxide also decreased expression of specificity protein-1 (Sp1), Sp3 and Sp4 transcription factors and this was consistent with downregulation of the Sp-dependent genes survivin, bcl-2, VEGF and VEGFR1. Similar results were observed for a structurally-related triterpenoid, methyl 2-cyano-3,12-dioxooleana-1,9-dien-28-oate (CDDO-Me), which is currently in clinical trials. Both CDDO-Me and CDDO-Me decreased mitochondrial membrane potential and induced reactive oxygen species (ROS), and these responses were also critical for triterpenoid-induced downregulation of Sp proteins which was inhibited by the antioxidants dihydrothreitol and glutathione. This demonstrates a common mechanism of action for CDDO-Me and CDDO-Me which is due, in part, to mitochondrial toxicity.

**533 HEPATOCARCINOGENIC PHENOBARBITAL TREATMENTS ARE ASSOCIATED WITH EARLY, PERSISTENT ALTERATIONS IN THE EXPRESSION OF THE MIR-200 FAMILY IN THE LIVER OF MALE RAT.**

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MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate protein levels. Evidence has accumulated in recent years highlighting the importance of miRNAs in liver development, function, and pathology. Therefore it is important to identify
the role of miRNAs in the adaptive and toxic responses of the liver to chemical carcinogen exposure. Using microarrays we showed that a carcinogenic dose (1000 ppm) of the non-genotoxic rat carcinogen, Phenobarbital (PB) resulted in distinct liver miRNA profiles compared to the two non-carcinogenic doses (50 and 500 ppm) after 14 days dietary dosing. Among the miRNAs uniquely altered by the carcinogenic dose at 14 days was miR-200b. The expression of this miRNA was unaffected by all doses at the earlier time points examined, 1, 3, and 7 days dietary dosing. Real-time PCR also confirmed this specific upregulation of miR-200b by the 1000 ppm dose at 14 days. Additionally miR-200b was still upregulated in the livers of animals exposed to 1000 ppm PB for 90 days dietary dosing, as quantified using real-time PCR. Western blots confirmed the downregulation of two proteins which are known targets of miR-200b, the Zeb1 and Zeb2 transcription factors. These proteins have been implicated in regulation of DNA damage-induced apoptosis, cell cycle arrest, and drug-resistance. Furthermore, by repressing epithelial markers, such as E-cadherin, and inducing mesenchymal markers Zeb1 and Zeb2 are master regulators of epithelial to mesenchymal transition (EMT), a process involved in cancer progression and invasion. Interestingly early, persistent deregulation of members of the miR-200 family has been reported previously in response to the seemingly unrelated carcinogenic treatments of acetaminofluorine and Choline-deficient L-amino Acid Defined diet. Collectively these data indicate that members of the miR-200 family are involved in the liver response to carcinogenic stress, the effect possibly mediated via the Zeb1 and Zeb2 transcription factors.

**S 534 IDENTIFICATION OF NON-GENOTOXIC CARCINOGENS USING NMR-BASED METABOLIC PROFILES AND BAYESIAN SIGNAL PROCESSING.**

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Identification of non-genotoxic carcinogens presents a considerable challenge because in vitro mutagenesis tests do not detect nongenotoxic carcinogens, two-year in vivo continuous exposure rodent studies are costly and time-consuming, and the early markers that are currently used (increased organ weights, xenobiotic enzyme induction and histopathology) are inadequate. A better method for predicting non-genotoxic carcinogenicity would therefore be highly desirable.

Metabolomics aims to understand biological processes by examining the metabolic changes associated with them. We increased the utility of high resolution 1H NMR spectroscopy-based metabolomics by using advanced Bayesian modelling of the time-domain 1D NMR signal to improve information recovery from spectra. This approach was applied to study non-genotoxic hepatocarcinogens in male F344 rats continuously exposed to ten different compounds for up to 91 days. The compounds were dosed at either the carcinogenic dose or the maximally tolerated dose. The major trend in the NMR dataset was separation according to presumed mode of action: that is compounds that are agonists for peroxisome proliferator activated receptor alpha or inducers of the cytochrome P450 2B clustered together in a Principle Component analysis. We then tested the ability of Predictive Multivariate Discriminant Analysis models, which were built based on the Bayesian deconvolution of aqueous liver samples obtained at the carcinogenic dose of phenobarbital in samples generated in a separate in vivo study and NMR spectra generated on a different NMR machine. The results showed improvement in the predictive classification of the carcinogenicity in this second phenobarbital dataset that suggests it is possible to identify a characteristic carcinogenesis signature, even when it is masked by predominant the Mode of Action effects.

**S 535 TIMING MATTERS IN PREVENTION OF BENZO(A)PYRENE (BAP)-INDUCED APC<sup>MIN</sup> MICE COLON TUMORS BY RESVERATROL (RVT).**

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Diet and environment are implicated in the development of sporadic colon cancers. Our laboratory has already reported the development of colon tumors in <i>Ap<sub>Min</sub></i> mice orally administered with BAP. In this study we wanted to examine the timing of RVT administration on BAP-induced colon carcinogenesis in <i>Ap<sub>Min</sub></i> mice. The mice were grouped under 5 treatment categories. For Group I, only BAP was administered (in peanut oil) at a dose of 100 μg/kg via oral gavage over a 60 day period. For Group II, RVT (in 10% ethanol + 90% deionized water) was co-administered with BAP at a dose of 45 μg/kg. For Group III, RVT was administered for 1 week prior to BAP dosing. For Group IV, RVT was administered for 1 week post BAP dosing. The V<sub>th</sub> group received RVT only. Blood, jejunum, colon and liver samples were collected at the end of exposure; adenomas in the jejunum and colon were counted and subjected to histopathology. RVT significantly inhibited the development of adenomas in the jejunum and colon by 45 and 40 % (P < 0.001) respectively in Group II, compared to Group III (0 and 5%) and Group IV (0 and 2%). Though no significant difference was noted in the size of adenomas in jejunum of BAP + RVT-treated mice compared to that of the non-carcinogenic dose of phenobarbital (50 and 500 ppm) after 14 days dietary dosing. Among the miRNAs uniquely altered by the carcinogenic dose at 14 days was miR-200b. The expression of this miRNA was unaffected by all doses at the earlier time points examined, 1, 3, and 7 days dietary dosing. Real-time PCR also confirmed this specific upregulation of miR-200b by the 1000 ppm dose at 14 days. Additionally miR-200b was still upregulated in the livers of animals exposed to 1000 ppm PB for 90 days dietary dosing, as quantified using real-time PCR. Western blots confirmed the downregulation of two proteins which are known targets of miR-200b, the Zeb1 and Zeb2 transcription factors. These proteins have been implicated in regulation of DNA damage-induced apoptosis, cell cycle arrest, and drug-resistance. Furthermore, by repressing epithelial markers, such as E-cadherin, and inducing mesenchymal markers Zeb1 and Zeb2 are master regulators of epithelial to mesenchymal transition (EMT), a process involved in cancer progression and invasion. Interestingly early, persistent deregulation of members of the miR-200 family has been reported previously in response to the seemingly unrelated carcinogenic treatments of acetaminofluorine and Choline-deficient L-amino Acid Defined diet. Collectively these data indicate that members of the miR-200 family are involved in the liver response to carcinogenic stress, the effect possibly mediated via the Zeb1 and Zeb2 transcription factors.

**S 536 ALTERATIONS IN REGULATORY T CELLS: NOVEL PATHWAYS TO IMMUNOTOXICOLOGY.**

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Regulatory T cells (T(Reg)) have been shown to be critical in the maintenance of immune responses and T cell homeostasis. For example, depletion of CD4(+)CD25(+) T(Reg) from mice resulted in the development of multiorgan autoimmune diseases. CD4(+)CD25(+) T(Reg) and/or IL-10-producing Tr-1 cells are capable of modulating cell signaling thereby suppressing or attenuating Th2 responses to allergens. Moreover, adoptive transfer of CD4(+)CD25(+) T(Reg) from healthy to diseased animals resulted in the prevention or cure of certain autoimmune diseases, and was able to induce transplantation tolerance. Clinical improvement seen after allergen immunotherapy for allergic diseases such as rhinitis and asthma is associated with the induction of IL-10 and TGF-beta producing Tr-1 cells as Treg function (such as Foxp3 expressing IL-10+ T-cells), with resulting suppression of the Th2 cytokine signaling pathways and products. Activation, expansion or suppression of CD4(+)CD25(+) T(Reg) in vivo by xenobiotics, including drugs, may therefore represent a relevant mechanism underlying immunotoxicity, including allergic asthma, autoimmune disease, and immunosuppression.

**S 537 INTRODUCTION TO THE ROLE OF TREGS IN IMMUNITY.**


Central or deletional tolerance in the thymus is incomplete. Therefore, mechanisms of peripheral tolerance are vital to protect from clinical autoimmunity mediated by autoreactive T-cells seeded into the peripheral immune compartment. It is clear that the normal mechanisms of peripheral tolerance are insufficient to prevent autoimmune disease and mechanisms of dominant tolerance mediated by regulatory T-cells (T-reg) are required. Defective regulatory T cell functions have been linked to the breakdown of immunologic self-tolerance in patients suffering from autoimmune diseases. Recent advances in the investigation of the generation and function of T-reg have improved our understanding of dominant peripheral tolerance. The use of reporter mice in combination with new methods to track antigen-specific T-cells in vivo have helped explain T-reg-mediated regulation of immune responses to self and foreign antigens. However, the molecular and cellular mechanisms by which T-reg cells are induced have not yet been fully elucidated. T-reg express Foxp3, a transcription factor that is required for their development and regulatory function. It has been suggested that T-reg function is modulated by the cytokines IL-6 and IL-23 present in inflamed target tissues. The mechanisms by which T-reg inhibit induction of autoimmunity and how their function may be modulated by specific environmental agents and contribute to immune-mediated disease is an active area of investigation.

**S 538 ROLE OF IMMUNOREGULATORY CELLS IN CHEMICAL AND PROTEIN ALLERGY.**

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The mucosal immune system of the gut, also referred to as the Gut Associated Lymphoid Tissue (GALT) is highly understood in chemical and protein allergy, despite the fact that it is thought to contain by far the largest amount of all immune cells in the body. The GALT has the difficult task of protecting the organism from microbial intruders, while at the same time tolerating food constituents and a wide
variety of more or less reactive chemicals, including drugs. Bypassing or breakdown of oral tolerance may result in food allergy and may also be involved in drug hypersensitivity. Mucosal tolerance is maintained by the interaction of antigen-presenting cells and regulatory T cells. In recent years, regulatory T cells have emerged as a key player in the immune system, playing a crucial role in maintaining oral tolerance and preventing the development of food allergy.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a widespread environmental contaminant that potently suppresses adaptive immune responses in mice. TCDD-treated mice are more susceptible to several infectious diseases, but, at the same time, are protected from the development of certain autoantibody diseases as well as allergic disorders. We recently discovered that activation of the transcription factor, Aryl Hydrocarbon Receptor (AHR), by TCDD in antigen-stimulated CD4+ T cells appears to affect their differentiation away from Th1 helper cells toward immunosuppressive regulatory T cells (Tregs). The absence of Foxp3 expression in the Treg-like cells suggests that AHR signaling in activated T cells represents a novel pathway for the induction of Treg that may be amenable to manipulation for therapeutic benefit. Altered expression of several different genes have been associated with Tregs, such as the expression of IL-10, TGFβ, CD39, IL12RB2, as well as several other genes not yet known to be involved in Treg development or function. This presentation will discuss the results of current studies that are examining changes in gene expression induced by TCDD in CD4+ T cells when cultured under conditions that drive specific T cell differentiation pathways. Understanding the pattern of changes in gene expression will provide greater insight into the signaling pathways that drive AHR-dependent Treg development and the underlying mechanisms for TCDD's potent immunotoxicity.

R. Ponce. Comparative Biology and Safety Sciences, Amgen, Seattle, WA.

Immunomodulation using biotechnology-derived therapeutics is an established modality in the treatment of infection, cancer, and autoimmunity. However, systemic administration of these agents has also resulted in novel toxicities that are incompletely understood and are often poorly modeled in experimental settings. This was tragically exemplified by the development of an acute cytokine release response among six healthy volunteers following administration of a novel experimental agent, TGN1412, targeting regulatory T cells. In the aftermath of this event, global regulatory bodies have reconsidered the nonclinical safety assessment for immunomodulatory therapeutics in support of Phase 1 studies. This presentation will briefly review the promises and challenges associated with developing alternative approaches for modulating regulatory T cells, the case of TGN1412, the revised guidance for the nonclinical and clinical assessment of such agents, the available tools for assessing the potential risks to human subjects treated with immunomodulatory biologies and needs for developing novel approaches.

D. J. Dix and R. Thomas. 1U.S. Environmental Protection Agency, Research Triangle Park, NC and 2The Hamner Institutes for Health Sciences, Research Triangle Park, NC.

Tens of thousands of chemicals and other man-made contaminants exist in our environment, but only a fraction of these have been characterized for their potential risk to humans and there is widespread interest in closing this data gap in order to better manage contaminant risk. Current practice of exposure estimation, toxicity testing, and risk characterization for environmental contaminants is too slow to support high quality science-based regulatory decisions for thousands of contaminants. We will address the various components required for performing rapid, quantitative, and high-quality risk characterizations on thousands of contaminants. Approaches and technologies well suited to address specific aspects of this process include high-throughput hazard assessments addressing the complex biology associated with environmental toxicity; using reverse dosimetry, pharmacokinetics, and biomonitoring equivalents to account for dose and exposure in evaluating high-throughput screening results; defining and quantifying the uncertainty associated with high-throughput data; and, finally, breaking from the current paradigm and using high-throughput chemical risk characterization for screening, prioritization, and other regulatory applications.

D. J. Dix. U.S. EPA, Research Triangle Park, NC.

High throughput toxicity testing provides detailed mechanistic information on the concentration response of environmental contaminants in numerous potential toxicity pathways. High throughput screening (HTS) has several key advantages: expense orders of magnitude less than animal testing; (2) direct study of human gene, protein and cell targets; and (3) hundreds to thousands of contaminants can be studied simultaneously. Quantitative HTS hazard assessment can thus identify potential mechanisms and pathways by which a contaminant can lead to specific adverse outcomes. EPA's ToxCast project is evaluating the use of HTS for understanding the types of molecular and pathway perturbations caused by environmental chemicals, and building initial predictive models of in vivo toxicity for prioritization and hazard assessment. To date we have tested 309 pesticide active and industrial chemicals in 9 HTS technologies. These include cell-free assays, as well as cell-based assays in a variety of human and rodent primary cells and cell lines. Both individual assays and composite assays for effects on genes and pathways demonstrated a broad spectrum of chemical activity at the molecular and pathway levels. Many expected interactions were seen in the data, including endocrine and xenobiotic metabolism enzyme activity. Chemicals show widely varying promiscuity across pathways, from no activity to activity in dozens of pathways. This diversity of behavior is seen even within well-defined chemical classes. This approach promises to provide meaningful data on thousands of untested environmental chemicals, and to guide more intelligent, targeted testing of environmental contaminants in the future. This abstract may not necessarily reflect Agency policy.

R. S. Thomas. The Hamner Institutes for Health Sciences, Research Triangle Park, NC.

With the recent efforts in toxicology to develop and validate high-throughput screening (HTS) assays to replace current animal tests or prioritize compounds for testing, one challenge has been to understand what the in vitro concentration values in these assays mean in terms of human dosimetry and exposure. To address this question, additional in vitro assays were developed for two critical determinants of pharmacokinetics - hepatocyte clearance and plasma protein binding. Hepatocyte clearance was measured using primary human hepatocytes and human plasma protein binding was measured using equilibrium dialysis. Computational in vitro-to-in vivo extrapolation methods and reverse dosimetry were then used to estimate human oral exposures required to produce steady state in vivo concentrations equivalent to in vitro EC50 values. In pilot studies using ToxCast Phase I chemicals and HTS results, the estimated human oral exposures in the most sensitive assays for many chemicals were significantly lower than the rodent low-effect levels. When relevant in vitro assays were selected for chemicals with known mechanisms of action, the estimated human oral exposures were much closer to the rodent values. These results highlight potential challenges in interpreting results from the HTS screens given the relative sensitivity of the in vitro HTS assays compared with the in vivo effects. The current approach is being extended to all the ToxCast Phase I chemicals and a much larger data set will be available to assess the exposure-dose-toxicity relationships in the 21st century approach to toxicity testing.

S. M. Hayes and L. L. Ayward. 1Summit Toxicology, L.L.P., Lyons, CO and 2Summit Toxicology, L.L.P., Falls Church, VA.

This presentation discusses the integration of existing biomonitoring and pharmacokinetic data into the evaluation and interpretation of the ToxCast assay results. Case studies are presented in which the tested and responding concentrations in vitro from the ToxCast dataset are compared to measured in vivo blood or plasma concentrations from population biomonitoring studies and to Biomonitoring
Equivalents (blood or plasma concentrations of chemicals that correspond with existing exposure guidance values such as Reference Doses, etc.). Such evaluations may contribute to the understanding of the sensitivity of the ToxCast assays as well as to the relevance of the tested concentration(s).

545 ACCOUNTING FOR UNCERTAINTY IN THE APPLICATION OF HIGH-THROUGHPUT DATASETS.


The use of high throughput screening (HTS) datasets will need to adequately account for uncertainties in the data generation process and propagate these uncertainties through to ultimate use. Uncertainty arises at multiple levels in the construction of predictors using in vitro HTS data. Many HTS assays may have an inherent level of variability deriving from the nature of the biological processes used in the assay. In addition, measurement processes used to translate a biological effect in vitro into a quantified datapoint introduces variability as well. Finally, predictors are typically statistical or machine learning models, informed by biology mostly through fitting to training sets. The structure of predictors is not typically informed by biology in any deeply meaningful way. Quantifying the magnitude and nature of the resulting uncertainty is important both for the use of such predictors for prioritization and for the design of studies to generate more reliable predictors. This presentation will discuss how variability introduced by biological and measurement processes leads to uncertainties in the output from high throughput screening (HTS) assays. The ideas presented are illustrated for a set of HTS assays and target toxicity data used in EPA’s ToxCast™ program. Simulation results inspired by the ToxCast™ data show how the uncertainty in HTS results is transformed into uncertainty about predictions using those results, and help to quantify the relative importance of variability at different levels of the data generation process to uncertainty of ToxCast™ predictions. This work was reviewed by EPA and approved for publication but does not necessarily reflect Official Agency policy.

546 PUTTING HIGH-THROUGHPUT CHEMICAL RISK CHARACTERIZATION INTO REAL-WORLD PRACTICE.

S. Baron. NCEA, EPA, Washington, DC.

Understanding of complex, intertwined environmental stressors and potential impacts on human health has grown tremendously in recent years. Basic and clinical sciences, however, have significantly outpaced risk assessment science. Insights into health and disease exist, but it is unclear how to incorporate this information into risk assessment with current methodologies. Risk assessment will have to address fundamental paradigm shift from a reliance on animal toxicology data derived primarily from rodent bioassays. The need for this shift is made more imminent by the challenges of applying new types of data stemming from advances in computational toxicology and the huge volume of data that will be generated from the European Union’s Registration, Evaluation, and Authorization of Chemicals (REACH) Program. High throughput data will need to be translated into knowledge to support science-based decisions in risk assessment. The areas where this knowledge is expected to have an impact is in defining toxicity pathways and informing risk assessors about the interpretation of multiple modes of action for toxicity, and providing insight into human variability in key pathways and human susceptibility. The impact of this information will be qualitative and quantitative. For this type of information to be incorporated quantitatively, numerous challenges exist, not the least of which is extrapolation from in vitro test systems to in vivo human health outcomes. However, the challenges are not insurmountable. The quantitative impacts of this new toxicity information will probably be seen in cases of chemicals lacking significant data sets but for which toxicity fingerprints can be developed from high throughput assay. These results may be used to derive estimates of relevant potency to chemicals that have much larger data bases and affect the same toxicity pathways. In addition, challenges in extrapolating from effective concentrations in vitro will require additional considerations in the development of environmental exposure estimates. This abstract was reviewed by EPA, but may not necessarily reflect Official Agency policy.

547 GENOTOXIC IMPURITIES IN DRUGS AND DRUG PRODUCTS: WHAT IS THE RIGHT WAY TO DEAL WITH IMPURITIES IN R&D VERSUS REGULATORY GUIDANCE?


The process of chemical drug synthesis necessitates the use of highly reactive starting materials and/or intermediates that have the potential to be present as low level residues within the final drug/drug product. These materials often have the potential to react with DNA (genotoxic), with adverse health consequences to humans. Additionally, the quality of starting materials used in drug manufacture is not regulated and may inadvertently introduce genotoxic contaminants in final product. To minimize inadvertent health risks, the European Medicines Agency (EMA) issued guidance in June 2006 and subsequently the U.S. Food and Drug Administration (FDA) issued draft guidance in December 2008 mandating pharmaceutical companies to closely monitor, evaluate, and mitigate risks associated with potential genotoxic impurities in drugs and drug products. The new genotoxic impurity guidance integrates and interject stringent requirements to Q3A, B, and C guidelines. As with all guidance the burden of developing appropriate strategies to implement such guidance remains with the scientists at pharmaceutical companies with practical considerations. To better understand this new guidance, attendees will be provided with current state of the science and regulations and approaches to identify potential impurities in drug/drug products; an overview of evolving strategies to determine genotoxic potential of identified impurities during product development cycle; and, an opportunity to discuss strategies to mitigate risks using case studies. To gain a balanced perspective, representatives from industry, regulatory agencies, and others from the expert scientific community will address these issues as outlined. To begin, a brief overview of the genotoxic impurities issue in drugs will be provided and followed by presentations on the historical development and implementation of this guidance around the globe. Finally, the panelists will discuss lessons learned from previously approved drugs, a now well known case of contamination of an approved drug, review of SAR software’s available to aid initial assessment, and the impact of regulation on the future of the drug development research and development process.

548 SCIENCE AND REGULATION OF GENOTOXIC IMPURITIES IN DRUG SUBSTANCES AND PRODUCTS.

D. Jacobson-Kram. Office of New Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD.

While the use of pharmaceuticals is always a balance of risks and benefits, the same is not true for impurities in pharmaceuticals: impurities convey only risk. A number of international guidelines and regional guidelines instruct drug developers and regulatory agencies on how to evaluate and control impurities in drug substances and drug products. These guidelines explicitly identify triggers for reporting, identifying, and controlling impurities. In addition, the guidelines provide direction on the assays that should be used to determine if impurities are genotoxic. New guidelines from the EMEA and FDA provide direction on how levels of genotoxic impurities should be controlled both in marketed products as well as in drugs during development. This presentation focuses on the science and regulation of genotoxic impurities in drug substances and drug products.

549 REGULATION OF GENOTOXIC IMPURITIES IDENTIFIED DURING CLINICAL DEVELOPMENT: A REGULATOR’S EXPERIENCE.


In batches of active pharmaceutical ingredients (API) impurities may be identified even when such batches are already used in clinical development. Current ICH impurities testing guidelines however are not intended to apply to new APIs in clinical development and therefore it remained unclear when and to what extent such impurities had to be qualified and what limits were regarded acceptable. Guidance is now provided by the recent EMEA and draft FDA documents. Usually an in silico evaluation for alerts for a genotoxic potential based on structural activity relationships should be provided. If critical structures indicative for potential genotoxicity are identified, further qualification using an Ames test is recommended to confirm or overwrite the in silico alert. The recent guideline documents have defined acceptable limits for genotoxic impurities (GTI) during clinical development according to the stage threshold of toxicological concern (TTC) approach. Using case studies, different scenarios will be described. One example will be a new potentially GTI was identified during an ongoing phase two clinical trial. The GTI was present at levels more than 10 fold above the staged TTC level. Assessment of all clinical and preclinical factors led to the decision that the phase two study should be continued as halting the trial would pose a higher potential risk for serious health effects for the patients than the limited exposure to the GTI. Before further clinical development however the GTI had to be limited to TTC levels by improvement in pharmaceutical development. The impurity profile of an API should be evaluated as early as possible in pharmaceutical development ideally before clinical development is started. This however might not always be completely possible as pharmaceutical development is normally not finalized at that time point. To assess the acceptability of a potential risk in such cases all relevant clinical and preclinical factors have to be considered carefully in the assessment. The overall benefit/risk ratio has to remain favourable for the patient.
QUALIFICATION STRATEGIES-GENOTOXICITY STUDY DESIGNS AND EVALUATION OF DATA FOR REGULATORY SUBMISSION: A CASE STUDY.

G. Krishna, Preclinical Toxicology and Pharmacokinetics, Enzon Pharmaceuticals, Inc., Piscataway, NJ

Review current regulatory guidance’s (FDA, CHPM, ICH) on types of genotoxicity studies required to support comprehensive evaluation of potential genotoxic impurities. The testing paradigms can vary significantly depending on the quality of the impurity and the positive or negative genotoxic outcomes at each stage of testing. This presentation will provide a review of testing paradigms using case studies from previously approved drugs, and shifts in testing paradigms due to impact of past experiences.

MOVING GENOTOXIC COMPOUNDS FROM TTC CONSIDERATIONS TO A PERMITTED DAILY INTAKE CALCULATION - HOW TO DO AND WHAT INFORMATION DO YOU NEED?

E. Gocke and L. Mueller, PRN/BST, Genetoxology Bldg 732/15, F. Hoffmann La Roche Ltd., CH-4070 BASEL, Switzerland.

The recent case of contamination of Viracept tablets with relatively high levels of ethyl methanesulfonate triggered extensive and successful studies to establish a threshold for mutation induction of this alkylating agent. Studies of this type and others have led to the FDA’s tentative daily intake (TTC) guidelines for impurities. This presentation will review these data and what would be needed for other types of genotoxic impurities.

THE ROLE OF DATA SHARING IN DEVELOPING (Q)SAR MODELS FOR THE EVALUATION OF GENOTOXIC IMPURITIES.

S. McDonald, Lhasa Ltd., Leeds, West Yorkshire, United Kingdom. Sponsor: S. Coepe.

The advent of guidelines on the genotoxicity of impurities from both the EMEA and the FDA has led to the routine use of (Q)SAR models in the evaluation of potentially genotoxic impurities within the pharmaceutical industry. (Q)SAR models are developed using several methodologies but all rely on what data is available and its quality. Industry holds a vast quantity of genotoxicity data for impurities and process intermediates, access to this data would enable the further development of (Q)SAR models to improve predictions for genotoxicity. This presentation will describe how proprietary data is being shared between organisations and show how that data is actively being used to validate and improve existing (Q)SAR models.

METABOLIC SYNDROME AND INCREASED SENSITIVITY TO DRUG-INDUCED LIVER INJURY (DILI): HISTORIC PERSPECTIVE.

G. B. Corcoran, Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy/Health Sciences, Wayne State University, Detroit, MI.

Over the last several decades, the fraction of overweight and obese individuals in the US has steadily increased to represent more than 60% of all adults. This so-called epidemic of obesity has been directly linked to increases in cardiovascular and liver disease, diabetes mellitus, and various cancers. The onset of obesity is often preceded by or accompanied by a constellation of clinical features that is now termed metabolic syndrome. This syndrome consists of metabolic abnormalities that are known to increase the risk for type 2 diabetes and cardiovascular disease, including hyperinsulinemia, elevated insulin-like growth factor I, dyslipidemia, hypertension, hepatic steatosis, and obesity. The latter has been mechanistically linked to drug- and chemical-induced pathologies, including DILIs and cancers by animal and epidemiological studies. There is growing evidence that obesity creates an environment that increases sensitivity to drug- and chemical toxicities. Obesity, and the pre-obese phenotype of metabolic syndrome, may contribute significantly to the risk of idiosyncratic forms of DILI, an adverse reaction that is not readily detected by current safety evaluation testing, and that has accounted for a high percentage of post-market drug withdrawals over the past 30 years. This presentation reviews historic findings showing that obese individuals are more prone to drug-induced damage to liver (acetaminophen, furosemide, alloxil alcohol) and kidney (gentamicin) and introduces non-clinical models, pharmacokinetic changes, and clinical findings describing the adverse effects of metabolic syndrome and obesity on drug safety.

IN VIVO MODELING OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN ZUCKER RATS FOR THE PURPOSE OF PREDICTING DRUG-INDUCED LIVER INJURY.

T. P. LaBranche, Drug Safety R&D, PGRD, Chesterfield, MO.

Human patients with an “invisible first hit” to their liver (e.g., hepatitis without an increase in serum ALT) are allowed to enroll in clinical trials. Because these patients are thought to be at an increased risk for drug-induced liver injury (DILI), the development and validation of an in-vivo model of NAFLD for studying (if not predicting) the safety of xenobiotics is warranted. 10-week-old Zucker Fatty and Zucker Lean rats were fed ad libitum either normal rat chow or a rat chow consisting of 60% kcal from fat, for 8 weeks. Zucker Fatty rats, regardless of diet, were found to develop obesity, hyperinsulinemia, glucose intolerance, hyperlipidemia and hepatic steatosis, thereby fulfilling the diagnostic criteria for Metabolic Syndrome. Quantitative RTP-PCR identified alterations in mRNAs encoding hepatic metabolic genes, including G6PDX, ACC2, FABP4, and ACDL. Interestingly, Zucker Lean rats on high-fat diet developed hyperinsulinemia and hepatic steatosis, but were still glucose tolerant, thereby indicating a compensatory state. These data illustrate the potential relevance of Zucker Lean rats fed a high-fat diet for 8 weeks to the human condition, and set the stage for validation following exposure to both known and suspect DILI compounds.

DEVELOPMENT OF A HEPATOCYTE CULTURE MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) FOR PREDICTING DRUG-INDUCED LIVER INJURY (DILI) USING ZUCKER RATS.

J.L. Davila, Drug Safety R&D, PGRD, Chesterfield, MO.

Hepatic steatosis is considered a benign symptom but it may render the liver more susceptible to DILI. The mechanisms how steatosis sensitizes the liver to injury is still unclear. In the present study, we established a primary cell culture model system and analytical methodology to study DILI reactions using Zucker rats. Primary hepatocytes, isolated from Zucker rats fed for 8 weeks with normal or high fat diet were cultured on Matrigel/Matrigel configuration for several days. Hepatocytes polarity and phenotype and viability were maintained for at least 6 days in cultures.
Increased lipid vacuoles were observed both in hepatocytes isolated from Zucker Lean rats fed with high fat diet (but not with normal diet) and in Zucker Fatty rats fed with either normal or high fat diet. Preliminary data indicate that hepatocytes, isolated from Zucker Fatty rat, regardless of diet, and from Zucker Lean rats fed with either normal or high fat diet, were more susceptible cytotoxicity in the presence of drugs than were Zucker Lean rats fed with normal diet. These preliminary data suggest that primary Zucker rat primary hepatocyte cultures have the potential to be used as an in vitro model to study DILI reactions associated with NALFD.

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Numerous drug-induced toxicities result from inter-individual variation in the ability to metabolize and eliminate drugs from the body. Although genetics plays an important role, the greatest source of variation comes from other environmental factors such as disease states. NALFD is a chronic condition that comprises a spectrum of pathophysiologic conditions ranging from simple steatosis to the more severe progressive steatohepatitis. Because the liver plays such a key role in the metabolism and disposition of so many drugs, any disease state (such as metabolic syndrome) that results in the functional impairment of the liver has the capacity to alter the fate of most drugs within the body. Our results suggest that several of the major drug metabolizing enzymes and transporters are altered in different stages of human NALFD, as well as in animal models of steatosis and NASH. The disposition and elimination of model substrates has been demonstrated in human patients in a similar pattern to that predicted using animal models. Translation of these findings to patients with NALFD is underway. Understanding the effect of NALFD on the functional activity of individual drug metabolizing enzymes and transporters could help predict the fate of a drug in patients with these diseases.

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The highly increasing prevalence of obesity and type 2 diabetes mellitus in the general population makes nonalcoholic fatty liver disease the most common diagnosis in every-day practices. Lifestyle changes (mainly exercise withdrawal and weight gain) have probably heightened the prevalence of non-alcoholic fatty liver disease. Mortality in patients with non-alcoholic fatty liver disease is significantly higher when compared with that of the same age-genre general population. The prevalence of acute drug-induced liver injury (DILI) in a specific setting was evaluated, assessing eventual interactions with pre-existing hepatic illnesses. The non-alcoholic fatty liver disease presence was an independent risk factor in determining drug-related acute hepatitis, with an odds ratio of 3.95 (95% confidence intervals: 11.48–1.35). Central obesity was relevant in every patient with acute toxicity. Alcohol consumption and drug association did not influence the acute drug-induced liver damage. Non-alcoholic fatty liver disease conveys a nearly fourfold increase in DILI risk in obese middle-aged patients. non-alcoholic fatty liver disease, characterized by mitochondrial dysfunction, could predispose to drug-induced hepatotoxicity that probably shares the same pathophysiological mechanism.

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Phthalates, a group of chemicals with many commercial uses (e.g., solvents, additives, plasticizers), have been associated with effects on the male reproductive system of laboratory animals following exposures during development and in adults. Studies have shown widespread human exposure to phthalates, and there are concerns for phthalate-related reproductive and developmental toxicity to humans. Since humans are generally exposed to mixtures of phthalates, rather than to single chemical entities, the importance of conducting a cumulative human health risk assessment has been recognized. We will highlight important reviews relative to the effects of phthalate exposures in laboratory animals and humans, and the use of biomarkers to quantify human exposure to phthalates, including for susceptible populations. The ground-breaking 2008 National Research Council (NRC) recommendations regarding the assessment of cumulative risk of human exposures to phthalates, and other chemicals, will be discussed. Finally, an overview will be provided that will address the conduct and status of phthalate risk assessment at the U.S. EPA including the consideration of cumulative risk in response to the NRC report.

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Phthalates are found in personal care products, medications, paints, adhesives, and medical products. Data are limited on the potential human health effects of phthalates, but in experimental animals, several phthalates have demonstrated toxicity. We have developed biomonitoring programs to assess human exposure to selected phthalates. One such program, the National Health and Nutrition Examination Survey (NHANES), conducted in the United States by the Centers for Disease Control and Prevention, is designed to collect data on the health and nutritional status of the general population. NHANES data can be used to establish reference ranges for phthalates and other chemicals, provide exposure information for risk assessment (e.g., set intervention and research priorities), evaluate effectiveness of public health measures, and monitor exposure trends. We have used state of the art analytical methods to analyze thousands of urine samples collected from NHANES participants. NHANES data have shown that in the general population, exposure to phthalates is prevalent. We have also observed differences in urinary concentrations of phthalate metabolites by sex, age, and race/ethnicity, all of which probably reflect lifestyle differences. But one NHANES limitation is its exclusion of persons under 1 year of age. Further, NHANES by design does not include population groups that might be highly exposed or the collection of urine from persons younger than 6 years. Therefore, phthalate biomonitoring efforts other than NHANES are needed. Such efforts should focus on a) identifying those metabolites best suited for use as biomarkers (e.g., oxidative metabolites); b) characterizing potential phthalate metabolite bioactivity in humans; c) improving understanding of phthalate metabolite toxicokinetics in different populations, with emphasis on fetal and neonatal exposures—when susceptibility to potential adverse health effects of phthalates may be highest; and d) studying targeted populations with known exposure source(s) to better relate internal exposure to potential health effects.

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Evidence of adverse human health effects from exposure to phthalates remains limited. This is primarily a result of a lack of epidemiologic data rather than evidence of a lack of effect. Despite the limited evidence on human health effects, there are data showing widespread human exposure to multiple phthalates, as well as other chemicals, that may act together to produce a common adverse outcome. In addition, there is evidence of unique high phthalate exposure sub-populations. Data from human studies on reproductive health effects will be briefly summarized and discussed from the perspective of how we can further our understanding of human health effects from exposure to multiple phthalates. Specific endpoints of interest include male reproductive tract development and reproductive function in relation to phthalate exposure. Novel strategies to study high exposure populations as well as to study multiple exposure situations will be discussed. Rather than offering firm conclusions, the talk will raise several issues for further discussion and thought. Finally, limitations of traditional epidemiologic study methods will be discussed. These include, choice of study population, assessing exposure at the appropriate exposure window and defining sensitive relevant health endpoints.

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ferentiation, and 3) how mixtures of phthalates behave when combined with other phthalates or with other toxicants? In the in vitro study we have examined the postnatal development of male rat offspring after in utero exposure to 1) pairs of AR antagonists, 2) pairs of phthalates, 3) phthalates with AR antagonists, 4) five phthalates, 5) seven chemicals (four pesticides and three phthalates), 6) ten chemicals (four pesticides and six phthalates) and 7) the potent Ah receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin plus a phthalate. We have also examined the effects of these chemicals on fetal male rat hormone levels and testicular gene expression levels. Results of these studies demonstrate that only Dose Addition models accurately predict the effects of these mixtures on male rat sexual differentiation. For example, when ten chemicals were administered in utero, 100% of the males displayed reproductive tract malformations as predicted by Dose Addition models whereas Response Addition models predicted that none of the males would be malformed. We propose that the regulatory framework for cumulative risk assessments should not be based upon common mechanisms of toxicity, as this under-predicts the effects of mixtures of chemicals with dissimilar mechanisms of toxicity. Rather, the framework should be based upon the disruption of common fetal targets or systems during development regardless of the mechanism of toxicity. This abstract does not necessarily reflect EPA policy. NTP, NIH/NIH Interagency Cooperative Research Agreement HHS Y1-ES-8014-01; EPA RW7592285-01-0.

This NRC Committee was asked to consider whether cumulative risk assessment should be extended to phthalates as well as approaches that might be used for that purpose and even more broadly with other chemicals. The Committee favored a cumulative risk assessment for phthalates, since humans are exposed to multiple phthalates all commonly associated with the phthalate syndrome that exhibits many features similar to the human testicular dysgenesis syndrome. In approaching this cumulative risk assessment, the Committee recommended a major shift in strategy that it also considered should be broadly applicable across chemical exposures. The basis of this strategy was a shift from the current focus of cumulative risk assessment on structurally/mechanistically-related chemicals (e.g., dioxin-like compounds, OPs) or chemicals with overlapping geographical use, to an approach that focuses on cumulative risks arising from chemical exposures that produce common adverse outcomes, i.e., shared physiological consequences, as per the common adverse effect of phthalates on male reproductive function. Since phthalate syndrome arises from androgens insufficiency, the Committee recommended that in a cumulative risk assessment for phthalates, other chemical and non-chemical factors that likewise lead to androgen insufficiency, regardless of the mechanism by which they produce androgen insufficiency, should be considered cumulatively with phthalates. Further, the Committee recommended that this strategy be applied to other common adverse outcomes, e.g., studying the cumulative impact of chemicals in combination that have individually been shown to result in IQ reduction, e.g., lead, methylmercury, PCBs. A focus on common adverse outcomes should actually facilitate and expedite the shift from single chemical to cumulative risk assessment as that purpose and even more broadly with other chemicals. The Committee recommended that only Dose Addition models accurately predict the effects of these mixtures on male rat sexual differentiation. For example, when ten chemicals were administered in utero, 100% of the males displayed reproductive tract malformations as predicted by Dose Addition models whereas Response Addition models predicted that none of the males would be malformed. We propose that the regulatory framework for cumulative risk assessments should not be based upon common mechanisms of toxicity, as this under-predicts the effects of mixtures of chemicals with dissimilar mechanisms of toxicity. Rather, the framework should be based upon the disruption of common fetal targets or systems during development regardless of the mechanism of toxicity. This abstract does not necessarily reflect EPA policy. NTP, NIH/NIH Interagency Cooperative Research Agreement HHS Y1-ES-8014-01; EPA RW7592285-01-0.

In the NRC reports Science and Decisions: Advancing Risk Assessment and Phthalates and Cumulative Risk Assessment: The Task Ahead the U.S. EPA is challenged to move towards cumulative risk assessment and away from chemical-by-chemical approaches to determining public health. The U.S. EPA has more than decade of experience in assessing cumulative risks and has recently released a significant resource documents on cumulative risk assessment. Despite the importance of cumulative risk assessments, there are major challenges that have limited the number of cumulative assessments performed. Cumulative risk assessments are population based, thus separate assessments are required for different populations. Site-specific information will play a critical role in such assessments. In any population the combination of exposures to chemical and non-chemical stressors varies from person-to-person and from moment-to-moment. These complexities affect both the determination of the cumulative risks and the contributions of any one stressor. Simulation modeling is expected to play a major part in the assessment of cumulative risk. Modeling provides an effective means of tracking and combining the impacts of exposures to stressors that occur by multiple routes and sources. These models include exposure, PBPK, and biologically-based dose-response models (BBDR) which become more effective when they are linked so that data and assumptions in exposure models are passed on to PBPK and BBDR models. This session will present a review of the technical issues for each step of the cumulative risk assessment process. Therefore it is important that we begin with an overview from an NRC committee member who authored the report and follow up with presentations from leading speakers on the various phases of the dose-to-response modeling process—exposure, kinetics, and dose-response. Finally, we will present a statistical model for the evaluation of the impact of cumulative risks that can assist in ranking or screening cumulative risks.

The recent National Research Council (NRC) report “Science and Decisions” concludes that the processes of regulatory risk assessment and the decision-making it supports are bogged down. Major chemical specific risk assessments can take longer than 10 years; uncertainty, inherent in the process, contributes to the gridlock. At the same time, communities disproportionately impacted by environmental exposures express concern that risk assessments are narrowly focused on a limited number of chemicals, typically from a single source, and ignore non-chemical stressors. Also a second NRC report, “Phthalates and Cumulative Risk Assessment,” notes the importance of cumulative risk assessment in evaluating the combined impact of multiple phthalates and other chemicals on male reproductive development. “Science and Decisions” recommends short and long term actions for implementing cumulative risk assessment, emphasizing those that will enhance its utility for discriminating among options for decision-making. They include the development of databases and default approaches to address non-chemical stressors and guidelines, and simplified analytic tools for timely screening assessments and to facilitate stakeholder involvement in assessment. Finally, community stakeholders often call for better assessment of cumulative environmental impacts, including considerations of livability, environmental degradation and community vulnerability. We present and reflect on NRC recommendations for cumulative risk assessment in the context of California’s efforts to develop a framework and tools for cumulative impact assessment.
The continuum of exposure across time relevant to the toxicology question; opportunities and considering simultaneous aggregate exposure; have outputs showing unit; encompass non-chemical modulation factors imposed as a consequence of sequence time in order, duration, periodicity and express it in the smallest required community and illustrate variability across variable time intervals; properly se-assessment on critical factors such as exposure routes, timing of exposure and identification of the health condition of those people as they are exposed. To enable modeling of cumulative exposure, the toxicologist should ask the exposure assessor to focus the assessment on critical factors such as exposure routes, timing of exposure and identification of the salient populations. We will discuss the basic/required elements including the need to:1.focus on exposure opportunities that are relevant to the community and illustrate variability across variable time intervals;2.properly sequence time in order, duration, periodicity and express it in the smallest required unit;3.encapsulate non-chemical modulation factors imposed as a consequence of the non-chemical stressors;4.have a coherent architecture showing exposure opportunities and considering simultaneous aggregate exposure;5.have outputs showing the continuum of exposure across time relevant to the toxicology question;6.provide each chemical its own exposure profile for all the time continuums.

Physiologically-based pharmacokinetic (PBPK) models facilitate the simulation of the target tissue dose of chemicals in organisms on the basis of the key determinants of uptake and disposition. Cumulative effects of chemical mixtures and non-chemical stressors on the key determinants of pharmacokinetics and tissue dose can be assessed using this modeling framework. This presentation will focus on the current state of knowledge regarding the development and application of PBPK models for assessing cumulative risks. The PBPK models for chemical mixtures are developed by linking models for individual chemicals via quantitative descriptions of the changes in physiological parameters, physicochemical determinants or metabolic clearance resulting from cumulative exposures. By accounting for the dose-dependent change in such key determinants, these models facilitate the simulation of the change in dose metrics of relevance to mixture risk assessment. In such a framework of tissue dose-based cumulative risk assessment then, it is feasible to use the results of PBPK modeling of the consequence of combined effects of chemical and non-chemical stressors. PBPK models for simulating change in internal dose during combined exposures have been developed for mixtures of aromatic hydrocarbon solvents (e.g., toluene, benzene, m-xylene and ethyl benzene), drinking water pollutants (e.g., trihalomethanes), and polychlorinated biphenyls. Similarly, the PBPK models have been used to account for the effects of non-chemical stressors. Examples of such an application include the consideration of temperature effects on the skin blood flow and permeability to contaminants in drinking water, as well as the effect of consumption of alcohol on physiological and metabolic determinants. Overall, by accounting for the quantitative effect of multiple chemical stressors and nonchemical stressors (e.g., temperature) on the physiological and biochemical parameters, the PBPK models allow scientifically-sound estimation of the magnitude of change in tissue dose and risk during cumulative exposures.

Information from sources such as the National Health and Nutritional Examination Survey indicate that humans are indeed exposed to a variable array of different compounds, regardless of the temporal relationship between exposures. Therefore, a concern for human health is the potential for developing untoward physiological outcomes related to the co-occurrence of mixtures of compounds in biological compartments. As such, developing concepts and methods to integrate qualitative and quantitative information regarding biological perturbations across chemicals, in association with population vulnerability factors such as diet, genetic background, and socioeconomic status, is paramount in the characterization of cumulative risk to human health. Current methods used in the integration of exposure and toxicity information for individual stressors, including how to assess toxicological interactions in cumulative mixtures are presented.

The justification for cumulative risk assessments is based on the concept that the total risk from exposures to multiple chemicals from multiple sources will always be higher than the risk from any single source of one chemical. The magnitude of the differences between the risks from individual sources/chemicals and the cumulative risks in exposed populations was investigated using simulation models. This research suggests that for the majority of populations, the largest risk from an individual source and chemical is a reasonable proxy for the total cumulative risk for the individual in a population who receive the highest risks. Specifically, the maximum risk from a single chemical/source made up more than 70% of the total risk for individuals in the top 1% of the simulated populations. This finding was found to hold for populations exposed up to 100 different source/chemical combinations and when source-specific risks were highly correlated. This finding can be viewed as an occurrence of the Pareto principle, which states that the magnitude of an effect, where the individual sources of the effect follow a right-skewed distribution, will be dominated by the contributions from a small number of sources. This analysis suggests that assessments of risk in a population on a source-by-source basis may be sufficient to determine the upper bound of the population’s cumulative risks. This would be done by multiplying the highest individual risk times a factor of 1.4 (1.70%). If this risk was acceptable, then the cumulative risk would likely be acceptable. Such an approach should be useful in screening out populations that are at low risk from cumulative exposures. While this research focused on chemical risks, the approach may also be relevant to non-chemical stressors where such stressors vary across individuals.

Chronic low level arsenic exposure is a major public health concern, since it has been linked to increased risks of cardiovascular and metabolic-related diseases in human populations throughout the world. Epidemiological studies that carefully address arsenic speciation in complex exposure analysis provide evidence of arsenic-related increases in atherosclerosis, stroke, and diabetes, even in the heterogeneous United States population. To identify the mechanisms for this disease promotion, recent in vivo animal studies have focused investigation on pathogenic responses and signaling in arsenic exposures that are in the low to moderate range of human exposures. These studies revealed that arsenic signals at multiple levels for systemic metabolic changes. Arsenic effects that result in diabeticogenic hyperglycemia include redox-dependent suppression of signaling for insulin release from pancreatic beta-cells and direct effects on glucose transport processes. Altered glucose and lipid signaling for cytokine secretion following arsenic exposures have been implicated in generating chronic inflammation. Arsenic effects on macrophages and liver endothelial cells that are responsible for lipid clearance and regulating lipid metabolism, as well as bulk removal of atherogenic modified proteins or glycosaminoglycans, may explain the role of the metalloid in disease promoting deposition of lipids in vessel walls and abdominal fat. Receptor mediated activation of NADPH oxidases is implicated as a rate-limiting step in signaling for inflammation and vascular remodeling following arsenic exposure. Together, these epidemiological and animal studies provide mechanistic insight into molecular pathogenesis of metabolic effects of low level arsenic exposures that may contribute to increased risk of cardiovascular disease and diabetes.
LOW-CHRONIC ARSENIC EXPOSURE: EPIDEMIOLOGIC EVIDENCE FOR CARDIOVASCULAR DISEASE AND DIABETES.


Epidemiologic evidence from Taiwan, Bangladesh and Chile supports a role for high chronic exposure to inorganic arsenic in drinking water (>100 μg/L) in the development of cardiovascular disease and diabetes. At lower exposure levels (<50, <10 μg/L), those that are relevant to many populations around the world including the US, epidemiologic evidence has been limited by the lack of individual measures of exposure, the use of non-standardized cardiovascular and diabetes outcomes, and the lack of adjustment for relevant confounders. Growing experimental and epidemiologic evidence, however, suggests that even at low exposure levels arsenic may have important cardiovascular and diabetes consequences. In a recent epidemiologic study conducted in a representative sample of US adults who participated in the 2003-2004 National Health and Nutrition Examination Survey (NHANES), we evaluated the association of inorganic arsenic exposure with the prevalence of type 2 diabetes. After adjustment for diabetes risk factors and markers of seafood intake, participants with type 2 diabetes had 26% higher total arsenic and 10% higher dimethylarsinate concentrations in urine than participants without type 2 diabetes. After similar adjustment, the odds ratios for type 2 diabetes comparing participants at the 80th vs. the 20th percentile were 3.58 (95% confidence interval 1.18, 10.83) for total arsenic and 1.57 (0.89, 2.76) for dimethylarsinate. There was no association between arsenobetaine, a marker of seafood organic arsenicals, and diabetes. Additional US population-based studies have found increased arsenic biomarker concentrations with gestational diabetes and with electrocardiographic abnormalities. While these studies are cross-sectional and temporality between arsenic concentrations and health endpoints cannot be ensured, these findings support the need for high quality prospective studies in human populations exposed to a wide range of arsenic exposure.

ANTITOXIC RESPONSE AND ROS SIGNALING IN ARSENIC-INDUCED IMPAIRMENT OF PANCREATIC BETA-CELL FUNCTION.

L. Yi, L. Fu, Q. Zhang, C. G. Woolf, S. Collins and M. E. Andersen.

There is growing evidence that chronic exposure of humans to inorganic arsenic, a potent environmental oxidative stressor, is associated with the incidence of type 2 diabetes (T2D). A key driver in the pathogenesis of T2D is the impairment of pancreatic beta-cell function, the hallmark of which is decreased glucose-stimulated insulin secretion (GSIS). In contrast to what has been a prevailing beneficial view of antioxidants in preventing beta-cell dysfunction in diabetes, our studies propose that in response to arsenic exposure, transcription factor Nrf2-mediated adaptive induction of endogenous antioxidant enzymes plays a pathophysiological role in beta-cell function. My talk will focus on the emerging evidence that reactive oxygen species (ROS) derived from glucose metabolism, such as H2O2, act as metabolic signaling molecules for GSIS in pancreatic β-cells. Particular emphasis is placed on the potential inhibitory role of endogenous antioxidants, which rise in response to arsenic exposure, in glucose-triggered ROS and GSIS. Our studies demonstrated that cellular adaptive response to arsenic-induced oxidative stress, such as Nrf2-mediated antioxidant induction, plays paradoxical roles in pancreatic beta-cell function. On the one hand, induction of antioxidant enzymes protects β-cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment of insulin secretion. On the other hand, the induction of antioxidant enzymes by Nrf2 activation blunt glucose-triggered ROS signaling, thus resulting in reduced GSIS. These two premises are potentially relevant to impairment of β-cells occurring in the high- and low-dose of arsenic-induced beta-cell dysfunction, respectively.

REGULATION OF GLUCOSE TRANSPORT MECHANISMS BY ARSENIC.

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The etiology of Type II diabetes remains an enigma. Although diet and exercise are recognized as important factors that contribute to the development of Type II diabetes, little consideration has been paid to environmental factors that may contribute to the disease. Recent epidemiological data indicate that arsenic in drinking water contributes to diabetes, but the mechanism underlying arsenic-induced diabetes is not known. We have shown that arsenic exposure in mice, at levels typically found in drinking water, causes symptoms typically associated with Type II diabetes. After a week of arsenic exposure, blood and urine glucose levels were elevated in the mice. In addition, blood insulin levels were elevated suggesting that arsenic in drinking water produced insulin resistance in mice. When the glucose transporter, GLUT4, was analyzed in skeletal muscle, mice that were exposed to arsenic showed a decrease in GLUT4 membrane translocation consistent with insulin resistance. To further characterize the mechanism by which arsenic modulates glucose homeostasis, mouse adipocytes were cultured and exposed to arsenic and cytokine expression was evaluated using cytokine arrays. Exposing 3T3-L1 adipocytes to 50 ppb arsenic resulted in an increase in cytokine expression, namely IL-6, which is known to modulate insulin signal transduction by inhibiting the insulin receptor substrate (IRS) protein. Studies currently underway are characterizing the regulation of IRS in both adipocytes, as well as skeletal muscle cells.
URANYL NITRATE INHIBITS LACTATE GLUCONEOGENESIS IN ISOLATED HUMAN AND MOUSE RENAL PROXIMAL TUBULES: A CELLULAR METABOLIC STUDY.

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As part of a study on uranium nephrotoxicity, we investigated the effect of uranyl nitrate in isolated human and mouse kidney cortex tubules metabolizing the physiological substrate lactate. For this, isolated human and mouse renal proximal tubules were incubated with various labelled lactates in the absence and the presence of uranyl nitrate. In the millimolar range, uranyl nitrate reduced lactate re- moval and gluconeogenesis and the cellular ATP level in a dose-dependent fashion. After incubation in phosphate-free Krebs-Henseleit medium with 5 mM L-[1-13C]-, or L-[2-13C]-, or L-[3-13C]lactate, substrate utilization and product formation were measured by enzymatic and NMR spectroscopic methods. In the presence of 3mM uranyl nitrate, glucose production and the intracellular ATP content were significantly reduced in both human and mouse tubules. Combination of enzymatic and NMR measurements with a mathematical model of lactate metabolism revealed an inhibition of fluxes through lactate dehydrogenase and the gluco- neogenic enzymes in the presence of 3 mM uranyl nitrate; in human and mouse tubules, fluxes were lowered by 20% and 14% (lactate dehydrogenase), 27% and 32% (pyruvate carboxylase), 35% and 36% (phosphoenolpyruvate carboxykinase), and 39% and 45% (glucose-6-phosphatase), respectively. These results indicate that natural uranium is an inhibitor of renal lactate gluconeogenesis in both humans and mice. Moreover, they show that the cellular metabolomic approach is a precious tool to evaluate the effects of nephotoxic compounds.

GENERIC DIFFERENCES IN THE EXPRESSION OF URINARY MARKERS OF INFLAMMATION AND OXIDATIVE STRESS.

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Activation of inflammatory and oxidative stress pathways and the onset of renal, cardiovascular, cerebrovascular and other systemic damage have been shown to be mechanistically linked. This study compares the expression of inflammatory, stress and vascular biomarkers in the urine of male and female donors. Specimens were assayed for aldosterone, total protein, creatine kinase, creatinine, c reactive protein, heat shock protein, microalbumin, myeloperoxidase, myoglobin, neutrophil gelati- nase associated lipocalin, proantriaritide peptide, vascular endothelial growth factor and interleukins 1 alpha, 1 beta, 6 and 10 using enzyme-linked immunosorbent assays. Many of these markers have been assayed exclusively in serum specimens without reference to gender specific differences. Urine specimens are non-invasive and may provide for qualitative clinical testing once appropriate normal ranges have been established. Increased expression of these markers is asso- ciated, in varying degrees of specificity, with vascular damage, oxidative stress and inflammation. Establishing a link between gender and biomarker expression in urine is the first step in developing a diagnostic tool that may permit rapid selec- tion of an appropriate clinical intervention. Our research suggests that significant gender based differences may exist in the expression of urinary inflammatory and stress biomarkers. This work has been supported in part by the Agency for Community Treatment and Services of Tampa.

URINARY KIDNEY INJURY MOLECULE-1 (KIM-1) AS A RENAL BIOMARKER IN GENTAMICIN (GEN)-INDUCED RENAL INJURY AND RECOVERY.

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This study was undertaken to determine and characterize the sensitivity of KIM-1 relative to that of existing biomarkers of acute kidney injury. The evolution and re- covery of renal injury was followed in male SD rats given Gen (0, 75, 150, or 300 mg/kg) for up to three consecutive days. Representatives from each group were sac- rificed at 11 time points over 45 days. Necropsy sampling included serum for blood urea nitrogen (BUN) and creatinine (sCr), urine for KIM-1, kidney for RNA extrac- tion, PCR, and histopathology evaluation. Urinary KIM-1 (uKIM-1) levels showed a significant dose-dependent increase over days 1 to 7 that peaked at day 7, and then returned to control levels by day 15. Changes in uKIM-1 preceded histopathology changes and were preceded by changes in KIM1 gene expression. Increases in BUN and sCr were lower in magnitude than those of uKIM-1. Decreases in elevated BUN and sCr levels preceded decreases in elevated uKIM-1 levels during recovery. A completely blinded pathology evaluation using a semi- quantitative scale of 0-5 showed a correlation of the severity of kidney injury with all biomarkers. Discrimination methods including receiver operating characteristics (ROC) analysis indicated that uKIM1 and renal KIM1 gene expres- sion significantly outperformed BUN and sCr for the detection of renal injury. Conclusions: 1) KIM-1 is a more sensitive biomarker for detecting acute renal in- jury than BUN and sCr during both evolution and recovery of injury; 2) KIM-1 ex- cretion into urine diminishes with tissue repair; 3) The correlation of KIM-1 excre- tion into urine with tissue injury was better during injury evolution than during repair and recovery.

METABOLIC ANALYSIS OF RAT URINE FOLLOWING ACUTE EXPOSURE TO PERFLUORINATED CHEMICALS.


Perfluorinated chemicals (PFCs), namely perfluorooctanoic acid (PFOA) and per- fluorooctane sulfonate (PFOS), represent an emerging class of persistent and bioac- cumulative compounds. Global occurrence of these fluorochemicals, coupled with probable human exposure, has prompted investigations of the biochemical impacts of PFCs that elicit toxicity through modulation of peroxisome proliferator-acti- vated receptors (PPAR) as well as other modes of action. Genomic studies have shown that PFOA and PFOS affect genes involved in cholesterol synthesis and fatty acid metabolism and result in signs of steatosis and hepatomegaly in rats. As a bio- marker-endogenous approach, this study focused on the use of metabolomics for identifying fluxes in the endogenous metabolome using proton nuclear magnetic resonance (1H-NMR) and both liquid and gas chromatography coupled to mass spectrometry (LC-MS* and GC-MS). To study this, male SD rats were dosed daily by gavage for 5 days with 20 mg/kg PFOA or 10 mg/kg PFOS. Urine was collected 24 hrs prior to, and twice daily during, the exposure period at 8 and 16 hr intervals. Urine was either buffered (H-NMR), filtered and diluted (LC-MS*), or extracted with chlo- roform:methanol, lyophilized and derivatized (GC-MS) prior to analysis. Spectra were subjected to principal components (PCA) and partial least squares discrimi- nant analysis (PLS-DA) to determine the effects of each PFC on the urinary metabolic profile. For each analytical platform, differences between the control and exposed groups were observed at the earliest time point. Moreover, PFC-related ef- fects were temporal and classes sustained distinct separation following three days of exposure. Components of the spectra responsible for time and PFC-dependent clustering are being investigated. Identification of significant changes in urinary metabolites will aid in identifying biomarkers associated with PPAR activation, he- patotoxicity, and exposure to these and other PFCs.

ROADMAP FOR NOVEL BIOMARKER CANDIDATE NOMINATION FOR PREDICTIVE SAFETY TESTING CONSORTIUM (PSTC) HEPATOTOXICITY WORKING GROUP.

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Arginase 1 (Arg1) and glutathione S-alkyltransferase Alpha (GstOa) are two novel biomarkers of liver toxicity. “Fit-for-purpose” ELISA assays have been validated and performance of these biomarkers has been assessed for specificity and sensitivity rela- tive to ALT over a number of studies. The data in support of, and strategy used to evaluate and nominate these biomarkers for potential Voluntary Exploratory Data Submission (VXDS) will be detailed as a guidance for future efforts in bringing candidate biomarkers forward. Roadmap milestones should include 1) clearly de- fined biological claims described in a Biomarker Research plan submitted to C-Path and the FDA; 2) mechanistic/biological rationale for the biomarker; 3) assay vali- dation; 4) sample/study assessment based on the biological claims; 4) performance evaluation relative to ALT and histopathology; 5) nomination within the PSTC Working Group; 6) independent validation from PSTC members; 7) VXDS sub- mission. The real world experience of Arg1 and GstOa will be used to illustrate the process.
582 IDENTIFICATION OF SENSITIVE URINARY Biomarkers FOR MULTITARGETED Receptor tyROSINE KINASE InHIBITOR-INDUCED GLomerULAR CHANGES.


Multi-targeted receptor tyrosine kinase (RTK) inhibitors are being developed as anti-angiogenesis agents for the treatment of cancer. RTK inhibitors could also lead to alterations in the glomerular ultrafiltration apparatus by disrupting the vascular endothelial growth factor (VEGF) signaling pathway in glomerular endothelial cells. The purpose of this study is to identify sensitive biomarkers for RTK inhibitor-induced glomerular changes. Male Sprague-Dawley rats were administered an experimental RTK inhibitor orally for 7 days to investigate the correlation of several urinary biomarkers with microscopic and ultrastructural changes. Glomeruli obtained by laser capture microdissection were also subjected to transcriptional and protein-based changes, using microarrays and antibodies targeting specific pathways. The results indicate that urinary albumin is a highly sensitive marker of the glomerular alterations associated with RTK inhibitors in rats.

583 THE ROLE OF IGf-1 IN THE MurINE SiLICOSIS MoDEL

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Prolonged exposure to crystalline silica in occupational and environmental settings induces chronic lung inflammation that can progress to fibrosis, i.e., silicosis. Despite existing standards in the workplace, silicosis remains a prevalent health problem throughout the world, particularly in developing nations. While silica is phagocytosed by macrophages and known to induce apoptosis, not all pulmonary macrophages die following exposure to silica particles. In the human and murine systems, macrophage subsets have been described. The present study hypotheses that a subset of pulmonary macrophages preferentially survives silica exposure and potentially plays a key role in the silicotic process. Previous work observed silica-induced changes in macrophage subpopulations in the murine lung. In addition, microarray data suggested a significant increase in insulin-like growth factor (IGF)-1 mRNA. While IGF-1 can activate fibroblasts, it also induces the Akt survival pathway. Assessment of lavage fluid following silica instillation found a significant increase in IGF-1 levels at multiple time points. While in vitro experiments were performed to confirm the link between IGF-1 and Akt activation, in vivo experiments using TH2-deficient mice further confirmed this link, as well as the potential role of IGF-1 in silicosis. At various time points following silica instillation, pulmonary macrophages were assessed for survival using flow cytometry, and a subset was discovered to have increased levels of IGF-1 activation. These results, combined with earlier studies, suggest that IGF-1 promotes survival of pulmonary macrophage subsets, and that following Akt activation and survival, there is an alteration in the phenotype as determined by surface marker expression. This work is supported by NIH grants RR-017670 and ES015294.

584 GLUCOSE AND INSULIN RESPONSE OF SUBCHRONIC DOSING OF ATYPICAL ANTIPSYCHOTICS IN RATS.


Use of atypical antipsychotic drugs, including clozapine, has been associated with metabolic derangements in patients including weight gain, glucose intolerance, and insulin resistance to overt diabetes mellitus. The objective of this study was to evaluate a novel method, the In Vivo Glucose Tolerance Test (IVGTT), for monitoring the effects of atypical antipsychotic drugs on insulin-mediated glucose uptake in rats. Our subchronic and acute studies were performed to aid in validating a predictive method, with advantages over the currently utilized hyperinsulminemic-euglycemic clamp assay, for accelerated screening of compounds for their potential to induce metabolic derangements in rats. The effects of clozapine was assessed in male Sprague Dawley rats fed a diet of moderate fat and high in sucrose for six weeks prior to and during the dosing period for abnormal glucose handling as measured by the Intravenous Glucose Tolerance Test (IVGTT) model. Animals were dosed orally for four weeks with vehicle, clozapine (10 and 15 mg/kg) or aripiprazole (2.5 and 7.5 mg/kg) and food consumption was monitored during the entire study. Aripiprazole was used for comparison because of its low association with metabolic abnormalities in the clinic. The animals underwent surgery for double cannulation during week three of dosing. Rats were fasted overnight before being placed in the automated system for blood sampling of pre-dose and out to 3 hours after dextrose administration. Both insulin and glucose were significantly elevated for both treatments of clozapine after oral administration and prior to the dextrose bolus. Aripiprazole had no physiologically meaningful effects on either insulin or glucose compared with control rats. These data show that the glucose intolerance and insulin resistance previously observed in acute studies is still present after 28 days of oral administration of clozapine.

585 EPIDERmal GROWTH FACTOR receptor signaling regulates adipose mass by affecting food intake.

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Obesity results from taking in more calories than needed for energy balance and can lead to longer exposure to lipotoxic toxins. Food intake and energy expenditure are regulated through a multi-organ system, determines body weight regulation. Signals relaying energy storage and satiety from the periphery are sent to the central nervous system (CNS) where they, along with other neuronal signals, maintain balance. Due to the worldwide rise in obesity and related complications such as diabetes, stroke, and cardiovascular disease, understanding the mechanisms associated with this disease is necessary. The epidermal growth factor receptor (EGFR) is involved in adipogenesis and may contribute in this regulation. Using diet-induced obesity mouse models, we found that inhibition of EGFR genetically with the Egfr<sup>−/−</sup> hypomorphic allele or pharmacologically with a small molecule inhibitor to the EGFR tyrosine kinase (AG1478) decreases adipose mass deposition due to a decrease in food intake. Therefore, to determine EGFR’s role in this phenotype, we deleted Egfr specifically in periphery (i.e., intestines and adipocytes) and in the CNS using the Egfr<sup>lox/lox</sup> conditional allele and the Villin-Cre, a p2p-Cre, and GFAP-Cre transgenic lines, respectively. All mice were fed a high-fat diet over three months. MRI, clinical chemistry, body weight, and food and water measurements were taken. Upon sacrifice, heart, liver, and fat depots were dissected, weighed, and stored at -80°C. Deletion of EGFR within the intestines with Villin-Cre or within adipocytes with p2p-Cre showed no effect on adipose deposition. However, ablation within the CNS using GFAP-Cre caused a significant reduction in adipose mass. Given this and our previous data, EGFR signaling is important in this balance of energy homeostasis within the CNS by affecting food intake, and possibly activity. The exact mechanisms by which this occurs have still to be determined, however we suspect changes in anorexigenic and orexigenic neuropeptides within the CNS are driving this phenotype.

586 CROSS-SPECIES TRANSLATION OF SEizure POTENTIAL WITH AN MGLU2/3 AGONIST PRODRUG (LY2140023).

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LY2140023 (LY) is the methionine prodrug of the potent mGlu2/3 receptor agonist LY404593 and is currently under investigation for the treatment of schizophrenia. Convolusions have been observed in rats, but not in monkeys, during treatment with LY. The convulsions in rats were dose- and time-dependent, but reversible upon withdrawal of treatment. In a 6-month study, convulsions were first observed in rats administered 750 mg/kg after 56 days and after 106 days in rats administered 300 mg/kg. None of the convulsions in rats were associated with lethality; however, a rat administered LY for 6 months (750 mg/kg) was observed to have minimal brain lesions. No brain lesions were detected in rats which did not have a convulsion or in any other rats which were not a convolution. The brain lesions are likely not the cause of convulsions but were due to the physiological changes associated with a convulsion. A rat 3-month EEG study showed that there was a progression of clinical signs and adverse changes in EEG which preceded overt convulsion. During the first 3 weeks, clinical signs included tremor and increased activity. Between weeks 3-7, a progression of clinical signs occurred that was accompanied by the first signs of adverse EEG sharp waves. During the final 4 weeks, signs progressed to include clinical convulsion and increased frequency, amplitude and duration of adverse EEG sharp waves. In contrast, in a 1-year monkey study which included multiple-scheduled measurements of EEGs, there were no convulsions or adverse...
Neuronal damage elicits responses from glia that can be modulated by high levels of stress hormones. We evaluated neuronal damage and the glial response following treatment of C57BL/6J mice with corticosterone (CORT) and kaic acid (KA). Male mice were implanted with 100 mg/21 day release CORT pellets. After 7 days mice received an intraperitoneal injection of saline or 25 mg/kg KA, were scored for seizures for 4 h, and were allowed to recover for 24 h. Brains were sectioned at 60 microns to allow 3-D evaluation of cellular morphology, and were analyzed for neurodegeneration by the cupric-silver stain, for microglia by Iba-1 and CD68 immunohistochemistry, and for astrocytes by GFAP immunohistochemistry. KA treatment caused neuronal damage that was especially evident in hippocampus, cortex, and thalamus. CORT pretreatment decreased oxytropic staining. In 3-D space, ramified microglial cells occupied a specific volume that contained processes from that cell only. KA treatment caused activation of microglia and initiated a phenotype characterized by amoeboid morphology, in an astrogial phenotype that comprised large vacuoles of ingested debris. In 3-D space, activated microglia were surrounded by a buffer space presumably formed as cellular processes retracted. Iba-1 staining was decreased in animals treated with CORT alone or with CORT + KA. CD68 immunostaining was not observed in control mice; however, KA treatment caused increased immunoreactivity that was dispersed throughout the cell body and processes. In CORT-treated animals, CD68 immunostaining was increased in control mice, however, KA treatment caused increased immunoreactivity that was decreased. Basal GFAP immunoreactivity was observed in control mice where astrocytes displayed long, thin processes. CORT treatment resulted in increased GFAP immunostaining that was prevented by CORT pretreatment. These data indicate that high dosages of corticosteroid decrease neuronal damage caused by KA and subsequent astro- and microglial activation.

PROTEIN ARRAY METHODOLOGY IMPROVES THE DETECTION OF IMMUNE RESPONSE AGAINST PATHOGENS IN ANIMALS USED IN TOXICOLOGY STUDIES.

A. Leon and T. Quinn. LAHS, BioReliance, Rockville, MD. Sponsor: E. Zabalka. Testing new drugs in animal models is an integral component in pre-clinical and clinical studies. The toxicology data obtained from the animal could be tainted by the untested presence of pathogens. Thus the data obtained from toxicology studies in animal models depend on the health status of the animals used. An animal whose health is compromised will generate data that are not related to the toxicity of the drug. This underscores the importance of knowing the health status of the animal before it is used in any toxicology study. A parameter used to decide if an animal can be used in a study is to determine if it is infected or has been infected by common species-specific pathogens. The assessment is done by detecting the presence of the pathogen or by detecting the animal’s immune response to it. Due to the important role played by these assays in support of the drug development process, an improvement of detection level is always desired. In this abstract we report an improvement in the detection of immune response to pathogens by using a protein array based technology, as compared to a traditional and commonly accepted method. Serum samples were tested in the protein array to determine the limit of detection of a sample. Serum samples were prepared at several dilutions and tested by ELISA and protein array. Samples were tested for the detection of antibodies against several viruses, including Hepatitis B Virus, Human Immunodeficiency virus, Simian Retrovirus, and Simian T-Lymphotropic virus. Cut off values were determined by analysis of ROC curves from populations of positive and negative samples. Comparison of results between both assays showed an up to 32-fold improve in antibody detection using the protein array. These results suggest that the protein array could detect antibodies against specific pathogens earlier than the ELISA, when the antibody levels are low. This is important because if infected animals are used in toxicology studies invalid data will be generated. This improvement increases the level of confidence in the observations obtained in toxicology studies.

EXPRESSION OF PHASE-I ENZYMES IN 17 MOUSE STRAINS – A TOOL FOR TOXICOLOGICAL RESEARCH.

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The toxicity of many chemicals depends on their biotransformation by phase-I enzymes to more or less toxic metabolites. To determine the in vivo role of an enzyme pathway in the toxicity of chemicals, inducers or inhibitors of a pathway, and/or genetically modified animals, are often used. Unfortunately, these models are often not ideal. It would be useful to have additional models which provide natural, reproducible variations in expression of an enzyme to understand its importance in the toxicity of a chemical. Therefore, the purpose of this study was to determine whether the mRNA expression of hepatic phase-I enzymes was variable enough in different mouse strains to alter the toxicity of chemicals. Livers of 9-week-old male and female mice were collected from 17 strains, and the mRNA expression of 5 cytochrome P450s (Cyp), cytochrome P450 reductase (CPR), 10 aldehyde dehydrogenases (Aldh), 8 carboxyesterases (Ces), and 2 paraoxonases were quantified. Five groups of genes could be distinguished based on their variance in 17 strains; such as genes which have 1.8 to 4-fold (10 genes, e.g. Cyp2e1), 4 to 6-fold (7 genes, e.g. Cyp1a2, Cyp3a11, CPR), 8-fold (2 genes), 20 to 30-fold (4 genes), or 30 to 60-fold (3 genes). In conclusion, there was sufficient variation in expression so that low, medium, and high gene expressing representative strains and/or genders could be selected, providing a natural in vivo model to determine the importance of a specific phase-I enzyme in the kinetics and toxicity of a chemical. (Supported by NIH grants ES005716, ES009649, ES013714, DK081461, RR029140.)

A MOUSE MODEL OF SEVERE HALOTHANE HEPATITIS BASED ON HUMAN RISK FACTORS.

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Halothane is an inhaled anesthetic that induces severe hepatitis in approximately 1 in 20,000 patients. The known risk factors for the development of halothane hepatitis include female sex, mature age, genetics, and multiple exposures. The mechanism of the severe halothane hepatitis is not entirely understood. We examined human risk factors for the ability to alter the sensitivity of mice to halothane-induced liver injury. To evaluate the influence of sex and age on halothane sensitivity, 4, 8, and 10-12 week old (woo) female and male BALB/c mice were treated with halothane (15 mmol/kg, ip), and alanine aminotransferase (ALT) activity was evaluated 24h later. The 8wo and 10-12wo female mice developed severe liver injury (ALT ~8000 and ~7000 U/L), whereas this response was milder (ALT~2000 U/L) in males of the same age and in younger mice of either sex. Livers from halothane-treated, 10-12wo female mice developed extensive centrilobular necrosis, inflammatory cell infiltrate, and steatosis within 12h of halothane exposure. This is consistent with the histological findings in livers from human patients with halothane hepatitis. To examine the influence of genetics on the sensitivity to halothane, two inbred mouse strains (BALB/c and C57BL/6) were exposed to halothane (5, 15, 30mmol/kg, ip), and ALT activity was evaluated 24h later. There was no hepato-toxicity at any dose in the C57BL/6 mice, whereas dose-dependent hepatotoxicity developed in BALB/c mice. No liver injury developed when 10-12wo female mice were exposed to isoflurane (5, 15, 30mmol/kg), an inhaled anesthetic with less idio-synthetic hepatotoxicity liability than halothane in humans. Therefore, this animal model based on human risk factors is characterized by reproducible, severe hepatitis from halothane exposure and lesions characteristic of those seen in patients who died from halothane hepatitis. (Supported by NIH grant GM075865.)

EVALUATING THE SENSITIVITY OF 6 DIFFERENT F1 HYBRID MICE FOR GENETIC SUSCEPTIBILITY TO IONIZING RADIATION.

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Heterozygous allelic variation introduced by outcross of female inosicogenic mice to B6.129-Tip53tm1Brd N12 deficient male mice was predicted to modify tumor phenotype, prevalence, and latency. To evaluate the effect of allelic variation intro-
duced by the genetic outcross of female isogenic mice (A/J, BALB/cJ, BTBR T+ tf/J, C3H/HeJ, DBA/2J, or 129S1.SvJemJ) to male B6.129-Tp53tm1Brd N12 homozygous null mice, we measured the sensitivity of these F1 hybrids to ionizing radiation. Survival rates, body weights, and total gross lesions at necropsy were evaluated. Male and female F1 hybrids (8 weeks of age) were exposed to 0, 3 or 6 Gy of ionizing radiation (12 mice per sex/exposure) using a Cesium irradiator. Body weight and clinical observations of the mice were recorded weekly for up to 39 wk. A full screen necropsy was performed on all mice. Spleen, thymus, bone marrow, and gross lesions were taken at necropsy, weights/volume determined, and tissues flash frozen for molecular biology studies. Onset of mortality began at 12-17 weeks post radiation exposure for all F1 hybrids. Female A/J, BALB/cJ, C3H/HeJ, and 129S1.SvJemJ hybrids appeared to be more sensitive than males, with deaths occurring a few weeks earlier. The observed morbidity occurred fastest in BALB/cJ and BTBR T+ tf/J outcross as compared to the other F1 hybrids. All F1 hybrids developed similar neoplastic and non-neoplastic profiles, with thymic tumors, splenic lesions, and lymph node enlargement most evident. In BALB/cJ, the most common neoplasms were malignant lymphomas and granulocytic leukemias followed by a variety of epithelial tumors (carcinomas). These preliminary data show a difference in genetic susceptibility of F1 hybrid p53 deficient mice to ionizing radiation induced tumor onset and death.

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592 OXIDATIVE STRESS STATUS AND RELATED MAPK SIGNALING IN H9C2 CARDIOMYOBLASTS EXPOSED TO CHOLESTEROL SECOALDEHYDE.

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3β-Hydroxy-5-oxo-5,6-secocholestan-6-al (cholesterol secoaldehyde or ChSeco) is a newly discovered oxyester known to be formed at inflammatory sites as a result of myeloperoxidase/H2O2/Cl− or singlet oxygen-mediated oxidation of cholesterol. Previous studies from our laboratory have shown that ChSeco induces oxidative stress in H9c2 cardiomyoblasts and thereby causes cytotoxicity. In the present study, we show that cardiomyoblasts exposed to ChSeco (0–5 μM; 6 h) have a dose-dependent decrease in the cellular catalase activity. Pre-treatment of cardiomyoblasts with N-acetyl-L-cysteine (NAC; 5 mM; 1 h) and apocynin (10 μM) did not restore the enzyme activity while diphenyleneiodonium chloride (DPI; 10 μM) partially mitigated the ChSeco-induced loss. ChSeco (10 μM) also reduced the cellular GSH levels as described earlier, and prior treatment with DPI (10 μM) marginally improved the GSH levels, whereas, apocynin (10 μM) caused a further decrease. Consistent with these results, we observed a dose-dependent increase in the stress activated kinase (SAPK/JNK) and pre-treatment with NAC (5 μM; 1 h) could not reduce the level of SAPK/JNK. Phosphorylated p38 increased over (6 h) period contingent upon ChSeco exposure. The NADPH oxidase inhibitor DPI was effective in bringing down the levels of phosphorylated p38. Down-stream transcriptional regulators ATF-2 protein was unchanged while c-Jun protein showed decreased expression due to ChSeco exposure. Taken together, it appears that ChSeco-induced oxidative stress results from activation of the NADPH oxidase system and that the increased oxidative stress status causes activation of p38 and JNK pathways not involving the transcription factors, ATF-2 and c-Jun.

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593 INHIBITION OF CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 ALTERS PHOSPHOLIPID PROFILES DURING CYTOSTASIS IN PROSTATE CANCER CELLS.

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Our previous studies demonstrated that inhibition of calcium-independent phospholipase A2 (iPLA2) induces cytostasis in human prostate cancer cells as well as activation of p53, p38, ERK1/2, EGFR and cell cycle arrest. iPLA2, has significant roles in the maintenance of cellular membranes as a provider of lysophospholipid acceptors used in the Kennedy Cycle. Thus, we used high performance-2D thin layer chromatography in tandem with electrospray ionization-mass spectrometry (HPLC-2D-TLC-ESI-MS) to test the hypothesis that iPLA2, inhibition, using the iPLA2-selective inhibitor bromoenol lactone (BEL), induces cytostasis in prostate cancer cells by altering cellular phospholipid profiles. Treatment of LNCaP and PC-3 human prostate cancer cells with 10 μM BEL for 6 and 24 hr resulted in time-dependent increases in the abundance of phosphatidylinositol (PtdCho) phospholipids containing polyunsaturated fatty acyl chains. In contrast, the abundance of PtdCho phospholipids containing saturated fatty acids decreased. Specific PtdCho species decreased by BEL treatment in LNCaP cells included 30:2, 30:0, and 32:0 PtdCho. PtdCho species increased in LNCaP cells in the presence of BEL included 36:4, 36:2, 39:1, 40:4 and 40:5 PtdCho. On the other hand, BEL treatment decreased the abundance of 30:0, 32:0 and 40:5 PtdCho in PC-3 cells, while increasing the abundance of 34:3, 34:6, 38:3 and 40:4 PtdCho. Further, BEL treatment significantly increased 38:1 and 38:0 phosphatidylethanolamine (PtdSer) and decreased 40:6 and 40:5 PtdCho in LNCaP cells. BEL treatment did not alter any PtdSer phospholipid studied in PC-3 cells, and had no effect on phosphatidylethanolamines in either cell line. These results demonstrate that iPLA2, inhibition alters the phospholipid profile of human prostate cancer cells, identify the specific phospholipids involved and suggest that alterations in these phospholipids may be involved in the mechanisms of cytostasis and cytotoxicity induced by iPLA2, inhibition.

594 ER-iPLA2 MEDIATES OXIDANT-INDUCED RELEASE OF FATTY ACIDS, PREVENTING ER LIPID PeroXIDATION AND CA2+ RELEASE.

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Oxidant injury is a major component of ischemia/reperfusion and toxicant-induced acute kidney injury. We showed that Ca2+-independent phospholipase A2 γ (iPLA2γ), present in both endoplasmic reticulum (ER) and mitochondria, protects renal proximal tubule cells from oxidant-induced lipid peroxidation and necrotic cell death. The role of ER-iPLA2γ in oxidant-induced lipid peroxidation, fatty acid release, and Ca2+ release in isolated rabbit kidney cortex microsomes was investigated. Micromolar concentrations of cis-parinaric acid, a marker of lipid peroxidation, and exposed to the oxidant tert-buty l hydroperoxide (TBHP) in the presence and absence of iPLA2γ inhibition, using bromoetanol lactone (BEL). TBHP induced lipid peroxidation and iPLA2γ inhibition potentiated the lipid peroxidation. Electrospray ionization-mass spectrometry was used to identify the lipid species released from microsomes. This approach showed that linoleic acid (18:2) and arachidonic acid (20:4), products of iPLA2γ-mediated cleavage of phospholipids, and their oxidized forms (18:2-OH, 18:2-OOH, 20:4-OH, 20:4-OOH) increased two-fold or greater after TBHP exposure and iPLA2γ inhibition blocked their release. To test the role of iPLA2γ in oxidant-induced ER membrane disruption, we developed an assay to monitor ER Ca2+ release using Ca2+ indicator, fluo-4. After ATP-dependent Ca2+ loading, micromolar were treated with TBHP in the presence and absence of BEL. TBHP caused ER Ca2+ release and iPLA2γ inhibition potentiated the ER Ca2+ release. Together, these data support the hypothesis that ER-iPLA2γ protects renal cells from oxidant-induced necrotic cell death by releasing unsaturated and/or oxidized fatty acids from ER membranes, thereby preserving ER membrane integrity and preventing ER Ca2+ release and loss of Ca2+ homeostasis.

595 ALDEHYDE DEHYDROGENASE 7A1 (ALDH7A1) IS A NOVEL ENZYME INVOLVED IN CELLULAR DEFENSE AGAINST HYPOPEROXIC STRESS.

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Mammalian ALDH7A1 is homologous to plant ALDH7B1 that protects against various forms of stress such as salinity, dehydration and osmotic stress. In addition, mutations in human ALDH7A1 gene cause pyridine-decarboxylase and folic acid-responsive seizures. Humans have ALDH7A1 expression in Chinese hamster ovary (CHO) cells attenuated osmotic stress-induced apoptosis caused by either increased sucrose or sodium chloride. Purified recombinant ALDH7A1 efficiently metabolizes a number of aldehyde substrates including the osmytne precursor, betaine aldehyde, lipid peroxidation (LPO)-derived aldehydes and the intermediate lysine degradation product, c α-aminoacidic semialdehyde (ASA). The crystal structure for ALDH7A1 was also determined and supports the enzyme’s substrate

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specifications. Tissue distribution of ALDH7A1 protein in mice reveals highest expression in liver, kidney and brain, followed by pancreas and testes. ALDH7A1 protein is found in the cytosol, nucleus and mitochondria, thus making it unique among the ALDH enzymes. Analysis of human and mouse cDNA sequences revealed mitochondrial and cytosolic transcripts that are differentially expressed in a tissue-specific manner in mice. In conclusion, ALDH7A1 is a novel ALDH expressed in multiple subcellular compartments that protects against hyperosmotic stress by generating osmolytes and metabolizing toxic aldehydes.

PL 596 AHR-DEPENDENT LIPID MEMBRANE REMODELING: AN EARLY STEP FACILITATING BENZO[α]PYRENE-INDUCED APOPTOSIS.

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Benzo[a]pyrene (BaP) often serves as a model to study the mutagenic and carcinogenic poly cyclic aromatic hydrocarbons (PAHs). Our previous works have suggested primordial role of the plasma membrane, more specifically the membrane fluidity, in BaP-induced apoptosis (Gorria et al., Ann NY Acad Sci, 2006). The plasma membrane has various microstructures that are important for its function, including the presence of cholesterol-rich-microdomains (CRM). By analysing CRM from rat liver F258 epithelial cells using immunofluorescence, lipid analysis, RT-PCR and western blotting, we found that BaP induced re-organization of membrane CRM via cholesterol depletion, fatty acid composition changes, and ganglioside GM1 redistribution. Studies with siRNA showed that the depletion of cholesterol was caused by down-regulation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase, HMGCR), as a result of BaP-induced aryl hydrocarbon receptor (AhR) binding and H2O2 formation. Addition of mevalonate, the product of HMG-CoA reductase, inhibited BaP’s early effects on the plasma membrane facilitating the triggering of apoptosis. In contrast, no effects on the classical initiation steps BaP-induced p53 phosphorylation and H2O2 production were seen. Intracellular pH measurements suggest that the remodelling plays a critical role in BaP-induced alkalization observed during the early phase of apoptosis. Our data provide evidence that BaP via AhR binding and H2O2 formation change the plasma membrane microstructure, thereby enhancing apoptosis.

PL 597 OXIDATIVE LIPIDOMICS OF GAMMA-RADIATION INDUCED LUNG INJURY.

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Oxidative damage has been suggested to play a significant role in pathogenesis of gamma-irradiation-induced lung injury. Endothelium is likely a preferred target for early irradiation induced damage and apoptosis. Oxidized phospholipids (PLs) participate in apoptotic signaling. Therefore, we performed oxidative lipidomics analyses of PLs in cells and animals after irradiation. C57BL/6N/ המכ female mice were subjected to total body irradiation (TBI) at doses of 5, 10 and 15 Sv and sacrificed 4h and 24h thereafter. We found that irradiation caused apoptosis as early as 4h after TBI, as revealed by caspase-3/7 activation. We demonstrated that the pattern of PL oxidation 4 and 24 h after irradiation is non-random and does not follow the PL molecular species containing CL and PS is associated with the execution of apoptosis in pulmonary endothelium involving both extrinsic and intrinsic pathways. However, signaling roles of phospholipid (PL) oxidation products in endothelial apoptosis in the lung have not been studied. We employed oxidative lipidomics approach to identify individual molecular species of PLs involved in apoptosis-associated peroxidation process in hyperoxic lung. C57BL mice were sacrificed 72h after exposure to hyperoxia (95% oxygen). We found that HALI induced apoptosis (as evidenced by caspase 3/7 activation) accompanied by non-random oxidation of pulmonary lipids. Two anionic PLs – mitochondria-specific cardiolipin (CL) and plasma membrane phosphatidylserine (PS) – were the major oxidized PLs in hyperoxic lung. ESI-MS analysis revealed the formation of several oxygenation products in CL and PS. Quantitative LC-MS analysis revealed significant decrease of CL and PS molecular species containing C18:2, C20:4, C22:6 and C22:5 fatty acids. When lung PLs were incubated with cyt c/H2O2 comparable pattern of PL oxidation was observed. Similar to HALI, exposure of mouse pulmonary endothelial cells (MLEC) to hyperoxia (95% oxygen) resulted in activation of caspase 3/7. Moreover, oxygenated molecular species were found in the same two anionic PLs – CL and PS - in MLEC exposed to hyperoxia. Furthermore, we documented significantly decreased content of CL molecular species containing C18:2 and C20:4 as well as PS molecular species containing C22:2, C22:5 and C22:4. Treatment of MLEC with mitochondria targeted radical scavenger GS-nitroxide, XJB-131, resulted in significantly lower oxidation of both CL and PS. We speculate that cyt c driven oxidation of CL and PS is associated with the execution of apoptosis in pulmonary endothelial cells thus contributing to HALI. Supported by NIH HL70755, HL094488.

PL 598 PHOSPHOLIPID (PL) OXIDATIVE METABOLISM DURING MACROPHAGE RESPONSE TO ENVIRONMENTAL AGENTS.

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Macrophages play a fundamental role during the clearance of environmental agents from the lung. During this process macrophages are activated to release inflammatory mediators and undergo apoptosis. PLs and their metabolites are involved in inflammation and cell death. However, no comprehensive studies, using contemporary research tools such as mass spectrometry (MS), addressing the oxidation or hydrolysis of PLs (phosphatidylethanolamine, PC, phosphatidyethanolamine, PE, phosphatidylserine, PS, phosphatidylinositol, PI, and cardioplin, CL) in macrophages are available at this time. Therefore, we conducted a quantitative characterization, using MS1 and fluorescence HPLC/Amplex Red assays, of the oxidative and phospholipase A2 hydrolysis of individual molecular species of major classes of PLs on silica, zymozan, or single wall carbon nanotube (SWCNT) exposed macrophages (Raw 264.7, J774, or primary from C57BL/6 mice). We report that following exposure to silica, SWCNT, or zymozan macrophages experience a rapid, and selective peroxidation of anionic PLs in a manner that is independent of their cell abundance (CL>PS>>PI). Oxidation of CL in response to silica is followed by the accumulation of the hydrolyzed moiety of CL, monolysophosphatidylcholine, and oxidized free fatty acids shortly after the release of cyt c, and the externalization of PS in the cell membrane. Subsequently, oxidation of the same species of anionic PLs was identified in lungs of C57BL/6 mice exposed to SWCNT. We concluded that anionic PLs are important mediators of the macrophage response to environmental agents and that they undergo a highly regulated metabolism during macrophage apoptosis. Supported by NIOSH OH008282, NORA 927000Y, NIH HL70755, HL094488.

PL 600 VALIDATION OF A NON-HUMAN PRIMATE TELEMETRY MODEL FOR ASSESSMENT OF CONTRACTILITY PARAMETERS.

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Following the implementation of ICH S7b Guidelines, the evaluation of cardiac safety of new chemical entities routinely includes potential adverse effect on hemo-dynamic, chronotropic and dromotropic effects. However, potential inotropic ef-
fected of new compounds are equally important to anticipate. Current development in technology now allows simultaneous evaluation of ECG, systemic pressure and Left Ventricular Pressure (LVP), a good indicator of myocardial contractility. The aim of this study is to validate a model of chronically instrumented non-human pri-
nate (NHP) for the assessment of inotropic effect in conjunction with the evalua-
tion of effect on blood pressure, heart rate (HR) and ECG. A positive (Pimobendan) and two negative inotropic drugs (Verapamil and Propranolol) were tested. Pimobendan induces a rapid increase in dp/dt Max and a decrease dp/dt Min and the LV Ejection Time, two other important representative parameters of contractility. Verapamil and Propranolol caused a short and rapid reduction of dp/dt Max and a rise of dp/dt Min and LV ejection time. Modification of HR (approx 50% decrease) was noted only after administration of Propranolol. No effects on blood pressure or ECG parameters were reported. In conclusion, whilst no effects on systemic blood pressure, HR and ECG recording were observed, modifications of contractility parameters such as dp/dt Max, dp/dt Min and LV Ejection Time were detected after administration of Pimobendan and Verapamil. Therefore, eval-
uation of inotropic parameters in safety pharmacology provides valuable informa-
tion for the detection of risk of contractility of new drugs.

**601 COMBINED CARDIOVASCULAR AND RESPIRATION ASSESSMENT IN THE CONSCIOUS GÖTTINGEN MINI-PIG FOLLOWING INHALATION ADMINISTRATION OF ALBUTEROL**


The minipig is increasingly being used as a species of choice for toxicological and pharmacological studies. We have gained significant experience in using the teleme-
tered minipig in safety pharmacology studies, administering test compounds via several different routes. In this study we assessed the cardiovascular and respiratory effects of inhaled administration of the beta-2-adrenergic agonist, Albuterol (Salbutamol) to provide a positive control. Telemetered Göttingen minipigs (ca. 22 kg) were acclimatised to custom designed facemasks over a period of 10 days on 8 separate occasions. Throughout the mask-wearing procedures, the minipigs were acclimatised to harnesses that were fixed to poles on restraint tables. Initial duration of mask attachment was 30 min, gradually increasing to 90 min by the end of ac-
climatisation. In addition to wearing the mask, the minipigs were also acclimatised to an initial airflow (7 L/min per minipig) for 15 min. To assess the impact of the inhalation procedures, Albuterol was administered as a liquid droplet aerosol with water used as the vehicle following completion of the acclimatisation process. The 0.35 mg/kg Albuterol delivered dose (mass median aerodynamic diameter of 1.7 μm) caused decreases in arterial blood pressures and concurrent heart rate increased with a maximum change of approximately 136%, at 0.25 h, along with shortening of the ECG lead II PR and QT interval (coinciding with the observed heart rate changes). These changes in haemodynamics lasted for up to 18 h. The responses to Albuterol were similar in magnitude to those observed previously in the beagle dog. Albuterol caused an increase in tidal volume resulting in a respective increase in res-
piratory minute volume (RMV) from approximately 4.0 L/min to 5.5 L/min and continued for at least 90 min post dose. The pre-dose RMV value was noticeably lower than that predicted by some published algorithms.

**602 ECG RECORDING METHODS IN DOG TOXICOLOGY STUDIES: WHAT IS THE REAL BENEFIT OF EXTERNAL TELEMETRY?**

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Over the last years, ECG recording methods in non-rodent toxicology studies have significantly changed. Standard ECG snapshot recordings (STD) with manual readings have been replaced by computerised systems and more recently by contin-
uous acquisition using external telemetry (EXT). Initially technically challenging, EXT is now used routinely to frontline electrophysiology assessments or compare Safety Pharmacology (SP) and Toxicology study designs. 39 preclinical projects were reviewed to compare the quality of data obtained with each method and evalu-
ate their potential to predict the adverse effects observed in SP telemetry studies. Baseline dog data (STD: n=291, EXT: n=177) were analysed for statistical power, i.e. to determine the threshold to obtain a statistical difference (p<0.05) with an 80% confidence level. Heart rates (HR) obtained with STD (Mean±SD: 124±26 bpm) were higher and more variable than with EXT (85±20 bpm). Using a group of 4 dogs, detection of a significant drug-induced tachycardia requires HR values of 175 bpm with STD (+41%) but only 124 bpm (+46%) with EXT. Both methods were equally powerful to detect corrected QT (QTcV). Van de Water method) or PR prolongations. Toxicology study reports (STD: n=39, EXT: n=10) were reviewed to extract ECG findings and compare them with those obtained in the corresponding SP telemetry studies selected as the reference. HR increases and QTcV prolongations were better predicted by EXT than by STD (90% vs. 72% and 70% vs. 60% respectively). EXT provides easy continuous recordings with sufficient sensitivity and could be used to characterise ECG findings during early toxicity studies, where high exposures and cumulative doses are assessed for the first time. The detection of adverse effects in these studies facilitates the optimisation of later study designs and decisions during candidate drug selection. Accordingly, EXT should be considered when defining or reviewing pre-clinical cardiovascular strategies.

**603 ASSESSMENT OF PULMONARY ARTERY HYPERTENSION BY COLOR FLOW DOPPLER ECHOCARDIOGRAPHY IN THE ANESTHETIZED MINI-PIG AND MONKEY.**

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Germain sur l’Arbresle, France and 2Cardiology and Echography Center, Marseille, France.

Pulmonary artery hypertension (PAH) is identified as life threatening for certain drugs such as anti-HIV drugs. Regulatory toxicology studies could benefit from investigat-
ing the potential risk of PAH induced by new chemical entities. Investigation of pulmonary artery pressure (PAP) by catherisation in patients is considered the gold standard for diagnosis. However, the invasiveness and the thromboembolic risk faced by patients had led to use color flow Doppler echocardiography (CFDE) as a first line diagnostic means. The non-invasiveness of this technique makes it particularly attractive for longitudinal assessment of PAH in regulatory toxicology studies. Functionally, an increase in PAP is associated with a regurgitant jet through the tricuspid valve, into the right atrium. The measurement of PAP by CFDE is calculated from the Bernoulli equation, using the maximal ve-
locity (Vmax) of the tricuspid jet. Accordingly, the current study investigated the feasibility of PAP measurement by CFDE in two animal models of different cardiac size and right ventricular structure, the isolaurane anesthetized minipig and cynomolgus monkey (n=2/species). An acute elevation of PAP was achieved by a 30 min intravenous infusion of U46619, a thromboxane-A2 receptor agonist with high pulmonary vasculature tropism. Five to 10 consecutive Vmax values were av-
eraged every 2 to 5 minutes and time-dependent changes in PAP were determined. U46619 induced a maximal hypertensive change of 29 (monkey) or 55-66 mmHg (minipig), which was reached 15 (monkey) or 25 min (minipig) after the onset of infusion. On cessation of infusion, PAP rapidly declined in both species. Changes in PAP can thus be non-invasively and reliably measured by color flow Doppler echocardiography in two preclinical species. It can be easily implemented in regulatory toxicology studies for longitudinal assessment of pulmonary artery pressure if an effect on this parameter is suspected.

**604 ECG ACQUISITION BY EXTERNAL TELEMETRY FOR TOXICOLOGY (ET®) IN FREELY-MOVING CYNOMOLGUS MONKEYS. COMPARISON WITH CONVENTIONAL, SNAPSHOT ECG IN CHAIR-
RESTRAINED ANIMALS AND VALIDATION WITH DOFETILIDE, A QT INTERVAL PROLONGING DRUG.**

J. Briffa1, E. Chalencon, C. Bory, P. Lege, S. Baudet and S. Milano. MDS Pharmacology Services, Saint-Germain sur l’Arbresle, France.

The relevance and reliability of snapshot ECGs collected during toxicity studies in the cynomolgus monkey, a preclinical species with noticeably fast-varying car-
diovascular parameters may be compromised by the stress generated by the need to
restrain the animal in a chair. Moreover, the short duration of snapshot ECG does not allow performing qualitative assessment of ECG traces. Bluetooth technology
represents an attractive alternative to record and transmit external telemetry col-
lected surface ECG in a large set of freely-moving animals.

Accordingly surface ECGs were recorded with our external telemetry for toxicity (ET®) system, based on the JET™ (DSI) technology, in six freely-moving cynomolgus monkey. The day before recording, each monkey was equipped with the JET™ device housed in a jacket. Four days after the ET® session, snapshot, paper based ECGs were recorded from the same six animals restrained in chair. In both configurations, a 6 lead ECG was recorded. ET® ECGs were analyzed with
Ponemah-F3 Plus (DSI) and paper-based ECGs were analyzed by manual reading with a calliper. In both configurations, ECGs were recorded after oral dosing with 0.05 and 0.2 mg/kg of dofetilide or vehicle (Latin square design), from 0.5 h pre-
dose to 24 h post-dose. Heart rate, RR, PR, QT, QTc intervals, and QRS complex

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duration were measured with each system at specific time-points. Prolonged QTc was observed at both doses but only the ET® system permitted to distinguish well-separated dose- and time-dependent effects of dofetilide.

Our external telemetry for toxicology ET® system allows reliable recording of ECGs in freely-moving monkeys included into regulatory toxicology studies. The improved sensitivity of this approach to detect subtle changes in ECG parameters compared to snapshot ECGs may prove to be a powerful tool for the detection of ECG-modifying properties of new drugs in toxicology studies (e.g. QT prolongation).

**PL 605** EVALUATION STUDY OF BLOOD PRESSURE MEASUREMENTS USING AN IMPLANTED PA-C10-TOX TRANSMITTER IN CONJUNCTION WITH JACKETED EXTERNAL TELEMETRY IN CONSCIOUS UNRESTRAINED Cynomolgus MONKEYS.


**Introduction:** Current techniques used to accurately determine arterial blood pressure (BP) in conscious, unrestrained monkeys require invasive telemetry. This study evaluated the functionality of an implanted miniature telemetry blood pressure transmitter (Model PA-C10-TOX, Data Sciences International) for the collection of BP measurements in conjunction with electrocardiographic measurements using a jacketed external telemetry (JET™) system in conscious, unrestrained cynomolgus monkeys. Although animals were randomly assigned to groups in a parallel study design. Animals were given a single intravenous dose of Nω-nitro-L-arginine methyl ester (L-NAME), an eNOS inhibitor, at 0, 0.1, 1, or 10 mg/kg. On an alternate week, animals were given a single intramuscular dose of clenolide hydrochloride, an α1, agonist, at 0, 0.01, or 0.05 mg/kg. Undisturbed telemetry BP data were continuously collected with Ponemah® software (DSI) for at least 24 hours following dosing, pooled by sex, and analyzed by repeated measured analysis of covariance. **Results:** L-NAME induced a significant dose-dependent decrease in heart rate (-27 bpm) and a significant increase in mean arterial pressure (+8 mmHg). Clonidine induced a significant decrease in heart rate (-35 bpm) and a significant decrease in mean arterial pressure (-11 mmHg). Previous evaluation indicated substantial BP variation and drift in a male and a female cynomolgus monkey. The bi-weekly percent changes varied considerably between measurements from week 1 to week 12; ranging from -8 to 32 and -12 to 14 for systolic pressures and -16 to 56 and -17 to 24 for diastolic pressures, in the male and female respectively. As a result, follow up studies were conducted to further evaluate the source of this variation.

The follow up studies consisted of two phases; Phase I (2 males and 2 females) and Phase II (9 males and 9 females) animals were implanted with transmitters (DSI, model PAC-10-Tox). The BP measurements were evaluated for variability and pressure drift. BP measurements were collected continuously by jacketed external telemetry (JET™) for at least 24 hours. In Phase I, BP data were evaluated weekly for 4 weeks and in Phase II, evaluation was conducted on weeks 4, 10, and 19 post-surgical implantations. BP data were averaged and grouped into light and dark phases. In Phase I, the weekly changes ranged from -1 to 22 mmHg for systolic pressures and -10 to 11 mmHg for diastolic pressures. In Phase II, the BP changes ranged from -16 to 4 mmHg for systolic pressures and -5 to 2 mmHg for diastolic pressures. Understanding the sources of this variability will be critical to the accurate analysis of chronic blood pressure effects of test compounds in repeat dose toxicology studies.
acterize the current state of the science as it relates to the impact of vitamin and mineral supplementation on human health; review the statutory and regulatory perspective on vitamin use from a safety perspective; assess the credibility of meta-analysis in the safety assessment of vitamins; and elicit the mechanisms of these interactions—prooxidant vs. antioxidant effects or beneficial vs. adverse effects.

610 THE EVOLUTION OF THE EXTENDED ONE-GENERATION STUDY DESIGN FOR AGRICULTURAL AND INDUSTRIAL CHEMICAL HAZARD IDENTIFICATION.
S. Marty, Toxicology & Environmental Research & Consulting, Dow Chemical Company, Midland, MI.
In 2006, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) recommended a new approach to the safety assessment of agricultural chemicals (36-37, 2006). This approach modified the testing required for agricultural chemicals and emphasized the use of pharmacokinetics in dose level selection. Among the most significant changes is the inclusion of an extended one-generation study design in which chemicals can be evaluated for effects on the developing nervous, reproductive, and immune systems. Since its inception in 2006, several laboratories have worked with the extended one-generation study design. Based on the experiences of these laboratories, modifications to the study design have been introduced. Furthermore, the extended one-generation study is being developed as an OECD test guideline with some additional design modifications. Therefore, the implementation has reached a critical nexus, where for the first time data are available to assess the practicality of the extended one-generation challenges. This roundtable session will present experiences with the extended one-generation study and opinions on its use from both laboratory scientists and regulators. The goal of this session will be to discuss the strengths and weaknesses of the extended one-generation study approach proposed for the hazard assessment of both agricultural and industrial chemicals.

611 CAN ANIMAL NEUROTOXICITY PREDICT HUMAN DYSFUNCTION?
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An important purpose of animal toxicity studies is to predict human disease, with the goal of minimizing the impact of chemical exposures on public health. Animal experiments can assert causal relationships between exposure and effect, characterize profiles of effects and, in conjunction with pharmacokinetic and empirical models, quantify dose-response relationships and their impact on public health. This discussion will evaluate the ability of animal models to predict effects on public health, including advantages and disadvantages of several approaches, by addressing the following. What aspects of an animal model enable one to predict impacts on human health? What information is needed to make such predictions quantitative? Can animal models account for differences in sensitivity to chemical toxicants? What is the role of behavioral and other whole-animal tests in toxicology in the 21st century? How can animal models facilitate the development of biomarkers of neurotoxicity? These questions will be explored using four cases in which animal models have revealed significant characteristics of exposure to neurotoxic chemicals.

612 WEIGHING COMPLEX DATA IN RISK DECISIONS: CONCEPTS OF EVIDENCE-BASED TOXICOLOGY.
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One of the most significant challenges facing toxicology today is how the regulatory community can incorporate complex mode-of-action information into science-based decision making. Toxicology has made significant progress in developing and promoting use of mode-of-action frameworks as tools to transparently organize complex toxicity datasets for regulatory evaluations. However, implementation of framework approaches continues to be hindered by a lack of understanding and agreement as to how to efficiently and effectively weigh the data used in ultimate decision-making, i.e., when is it known enough? The medical community has initiated the practice of Evidence-Based Medicine (EBM), a data evaluation approach that has been successfully used to improve translation of complex clinical information into effective medical practice. The principles and approaches of EBM offer significant opportunity for parallel application to complex problems in toxicology, and in recent years active discussion has emerged within the field of toxicology on the potential value of incorporating EBM into what has been termed Evidence-Based Toxicology (EBT). The exploration and discussion of EBT is of great importance to toxicology in general and the eliciting the mechanisms of these interactions—prooxidant vs. antioxidant effects or beneficial vs. adverse effects.

IS 613 HUMAN HEPATOCYTES DERIVED FROM EMBRYONIC STEM CELLS: A NEW TOOL FOR IN VITRO TOXICITY TESTING.
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In vitro methods for liver toxicity testing have seen poor acceptance as an alternative to animal testing because of low specificity and sensitivity to in vivo outcomes. Hepatocytes are highly differentiated cells with complex functions that are particularly difficult to be maintained over an acceptable timeframe. Recently, some advances have been made to improve hepatocyte longevity and functionality using 3D and co-culture technologies. However, these models rely on the continuous availability of human primary liver cells obtained from a limited number of human donors. As a result, good quality cells are in short supply for research purposes. Generating functional hepatocytes from pluripotent stem cells would provide a continuous cell pool capable of expansion. The use of human embryonic stem cells (hESC) and differentiation into mature cell lineages is a new and rapidly evolving area of research with a great promise in generating alternative in vitro models to study human toxicities. However, while considerable advances have been made in recent years to develop hepatocyte-like cells from embryonic and other stem cells, the application of hESC-derived hepatocytes in toxicology still faces several challenges. This session will describe methods, current and future applications of hESC-derived hepatocytes for the assessment of mechanisms leading to hepatotoxicity. An overview will be given on technology developments necessary to generate an hESC-hepatocyte model which mimic adult liver function in vitro, has adequate enzymatic/transporter expression and longevity in long-term culture, and is translatable into higher throughput screening applications for predictive toxicity and ADME testing. Novel support systems will be described which have improved functionality and longevity of hESC-derived hepatocytes.

IS 614 RECENT ADVANCES IN PULMONARY SURFACTANT TOXICOLOGICAL ASSESSMENT AND THERAPEUTICS.
Pulmonary surfactant is critical for proper respiratory function. The primary role of pulmonary surfactant is to reduce surface tension in the lung and prevent collapse of alveoli and distal airways, thereby preserving functional residual capacity and promoting gas exchange. Surfactants are a complex mixture of phospholipids and proteins. Phospholipids lower surface tension, while proteins play a critical role in a variety of functions related to respiratory health and development, including the further lowering of surface tension by maintaining the phospholipid monolayer. The introduction of surfactant replacement therapy (SRT) in the United States (U.S.) in 1990 for the treatment of respiratory distress syndrome (RDS) has led to reduced morbidity and mortality in preterm infants. There is evidence that surfactant dysfunction exists in other lung diseases, and clinical trials have been conducted investigating the use of SRT beyond the treatment of RDS. The initial exogenous pulmonary surfactant introduced in the U.S. was a blend of synthetic phospholipids. However, the exogenous surfactants currently available for therapeutic use all contain proteins extracted from animal sources, raising the possibility for the inclusion of prions and non-target substances into the surfactant. In addition, analyses of these animal-derived surfactants have revealed considerable variability in terms of purity as well as the concentration of the target surfactant proteins. These concerns in turn raise questions regarding the potential toxicity of exogenous surfactants.

IS 615 ANTI-DRUG ANTIBODY-MEDIATED TOXICITY IN NONCLINICAL TOXICOLOGY STUDIES: IMPACT AND RELEVANCE TO HUMAN SAFETY.
Immunogenicity is a unique property of biotherapeutics thus it is accepted that the administration of a biotherapeutic to humans or animals has the potential to elicit an antibody response against the drug if the biotherapeutic is perceived as foreign.
Most biologics are human-specific proteins or monoclonal antibodies and it is not unexpected that the administration of these drugs may result in the production of anti-drug antibodies (ADA). ADA responses are a common challenge during the conduct of nonclinical toxicity studies for biologics, and these responses can potentially affect the outcome and interpretation of a toxicity study. The impact of ADA on toxicology studies can vary having no effect, an alteration of the pharmacokinetic profile resulting in decreased/increased systemic exposure, an abrogation of the pharmacological activity, or neutralization of the biological activity of an endogenous protein that mediates a critical biological function. Another potential consequence of the production of ADA is ADA-drug immune complex formation with deposition in various organs and tissues. These immune complexes can result in significant inflammation and tissue damage with resultant organ dysfunction. A common example of immune complex-mediated toxicity is immune complex-mediated glomerulonephritis. Immune complex formation has also been associated with “anaphylactoid-like” hypersensitivity reactions and serum sickness. In addition, although rare, ADA have been associated with classical IgE-mediated acute hypersensitivity reactions and autoimmunity. These various ADA-associated toxicities can confound the conduct and interpretation of toxicity studies. This session will highlight case studies to explore potential ADA-mediated toxicities including hypersensitivity reactions and immune complex formation/deposition and impact on clinical development/safety will be discussed.

**616 INTRODUCTION: GENERAL REVIEW OF THE TYPES OF ANTI-DRUG ANTIBODY-MEDIATED RESPONSES THAT CAN OCCUR IN TOXICOLOGY STUDIES.**

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Most biotherapeutics are recombinant human proteins or monoclonal antibodies specific a human receptor protein. Therefore, it is not surprising that most biotherapeutics induce an anti-drug antibody (ADA) response in nonclinical toxicology studies. While some biotherapeutics may be more immunogenic in one animal species vs. another, ADA responses can occur in all types of animal species used in toxicology studies, including nonhuman primates. ADA responses to biotherapeutics is generally greater in animals than humans (due to the protein structural property of the biotherapeutic being recognized by foreign by the animal model used) and most often, animal models have a low predictive value, and can even overestimate the ADA response rates in humans. While analysis of ADA responses in animals may not be relevant in terms of predicting potential immunogenicity in humans, evaluation of ADA responses in toxicology studies can assist in the interpretation of the study results, as well as the design of subsequent toxicology studies. The types of ADA responses that can occur in toxicology studies can vary. Likewise, the consequences associated with the different ADA responses can differ, ranging from having no impact on the study outcome to resulting in life-threatening toxicities. This symposium will review the types of toxicities that are a result of ADA-mediated toxicities and can occur in toxicology studies, as well as discuss how these types of ADA-mediated toxicities can impact the outcome of a study and how the data translates to human safety.

**617 MECHANISMS OF ANTI-DRUG MEDIATED TOXICITIES OBSERVED IN NONCLINICAL TOXICOLOGY STUDIES AND IMPACT ON CLINICAL TRIAL DESIGN AND HUMAN SAFETY.**

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The development of anti-drug antibody (ADA) responses frequently occurs in animal toxicology studies in response to administration of biotherapeutics. The level of ADA response and the observed toxic effects can be different in various animal species and are not easily predicted. In some cases, low levels of ADA may cause more toxicity than higher levels, or a biotherapeutic may be more immunogenic in the nonhuman primate than in a rodent species. In other cases, the development of ADA will have no effect on drug exposure or pharmacological activity of the drug, yet under some conditions, leads to complications as severe as life-threatening toxicities. For example, anaphylaxis is an ADA-mediated toxicity commonly observed in animal toxicology studies that can lead to acute death following the administration of a biotherapeutic. Other ADA-mediated toxicities, such as glomerulonephritis and periarteritis, can result from the formation and deposition of antibody-antigen complexes in various tissues. Since the incidence of ADA responses is generally viewed to be greater in animals than humans, caution must be taken in translating the relevance of ADA-mediated toxicities occurring in animal studies to human safety. This includes a comprehensive investigation of the etiology and the pathology of the ADA-mediated lesions, as well as also determining if the lesion is target associated or solely related to the formation of ADA. Although relevance to the clinic may be considered low, nonclinical study findings related to ADA may drive additional clinical monitoring until ADA formation in humans is better understood. This is particularly critical in cases where ADA may impact endogenous protein function. Without a thorough understanding in how the ADA-mediated toxicity occurring in animals truly translates to human safety, many biotherapeutic drug candidates could be prematurely terminated.

**618 ATYPICAL HYPERSENSITIVITY REACTIONS ELICITED BY A MAB TARGETING A HUMAN FC RECEPTOR.**


GMA161, an aglycosyl humanized monoclonal antibody that binds to hCD16A and hCD16B Fc-receptors, was evaluated for treatment of idiopathic thrombocytopenic purpura. A human double transgenic (Tg) mCD1616-/-, hCD16A+/-, hCD16B+ mouse was used for preclinical studies due to appropriate receptor expression and distribution. Following repeated doses of GMA161, acute and severe hypersensitivity reactions occurred and were accompanied by significant anti-GMA161 antibody titers. Similarly severe reactions were elicited by the murine surrogate, aglycosyl 3G8. Studies aimed at elucidating the mechanism of action behind these responses found that both transgenics were required to prompt severe hypersensitivity reactions and that anti-GMA161 antibody titers were significantly higher in mice with both transgenes compared to single Tg mice. These observations suggested that the severity of the reaction may be associated with the magnitude of antibody response. A second study further investigated two of the known pathways mediating murine systemic anaphylaxis: IgE – characterized by histamine release, a pathway commonly engaged when testing protein therapeutics in mice, and IgG – mediated by IgG binding to the CD16 receptor on macrophages and neutrophils and characterized by platelet activating factor (PAF) release. In this study, when the IgG pathway was blocked by pretreatment of a PAF antagonist Tg mice were tolerant of repeat dosing of GMA161 with no adverse clinical signs being observed. However blocking of the IgG pathway with an antihistamine administration did not reduce, and in some cases, exacerbated the hypersensitivity-like reaction. Anti-GMA161 antibodies resulted in significantly decreased exposure following repeated administration. In total, these studies indicate that the atypical hypersensitivity reaction following repeat dosing of GMA161 or aglycosyl 3G8 could be elicited by targeting CD16 receptors and exacerbated by excessive IgG formation by the second dose, and may be alleviated by inhibiting IgG rather than IgE-mediated hypersensitivity mechanisms.

**619 CASE STUDY ON THE IMPACT OF IMMUNOGENICITY ON ADVERSE EFFECTS IN TOXICOLOGY STUDIES AND CONSEQUENCES FOR THE CLINICAL DEVELOPMENT PROGRAM.**


Anti-drug antibody (ADA) responses can potentially affect the outcome and interpretation of a toxicology study and assessing the relevance of toxicity that may be ADA-mediated in animal studies to clinical populations can be difficult. During the development of a monoclonal antibody (MAB) drug which targets the immune system, low incidence adverse clinical and anatomic pathology findings were noted that were not immediately explainable as drug or ADA-related. In a 6-week cynomolgous monkey study with weekly dosing of 10, 75 or 300 mpk IV or 300 mpk SC, chronic periarteritis involving multiple organs was observed in 2/6 animals given 300 mpk IV, and in 1 tissue in 1/6 animals given 300 mpk IV. 1/6 animals given 75 mpk IV and in 1/6 animals given 300 mpk SC. The etiology of the vascular lesion was uncertain based on routine histopathology evaluation. A vascular target was not anticipated based on literature describing target-deficient humans or KO mice and was thus thought not to be related to the mechanism of action of the MABs. Evidence of an immune response to the MAB associated with enhanced clearance was noted in 3/5 animals with the lesion, and these animals tended to develop ADA quicker than non-affected animals that eventually became antibody positive. The safety margin at the NOAEL of 10 mpk was ~5x/20x based on AUC/Cmax, and FIH trials were initiated. In a follow up 3-month study with weekly SC dosing of 5, 25, or 90 mpk, periarteritis was noted in only 1 tissue of 1 animal at the mid dose. Immunohistochemical (IHC) staining for antihuman IgG and antimonkey IgG and IgM revealed granular staining in blood vessels with the vascular lesions of animals from both studies. This staining pattern suggested that ADA/drug complex deposits might have a role in the pathogenesis of the lesion. These data supported the conclusion that ADA contributed to the toxicity seen with this MAB and was not likely to be relevant to humans. Close monitoring of ADA in the clinical trials, along with other endpoints allowed for progression of single and multiple dosing.
CHARACTERIZATION OF POTENTIAL IMMUNE COMPLEXES OBSERVED IN CYMONOLGUS MONKEYS AND RELATIONSHIP TO OBSERVED TOXICITIES OF MONOCLONAL ANTIBODY DRUG CANDIDATES.

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Administration of monoclonal antibody drug candidates to cynomolgus monkeys produced lesions in various organs, including kidney, lung, heart and joint that could not be directly related to the pharmacological action of the compounds. The incidence and severity of the lesions across dose groups as well as histological character-acterization indicated possible immune complex formation and deposition. Several analyses were conducted in an effort to determine the role of immune complexes in the observed lesions. Key primary assessments included understanding the relationship between anti-drug antibody (ADA) status, drug exposure levels, and severity of lesions across dose groups and for individual animals in the toxicity studies. Immune complex deposition and formation was not always dependent on the dose of drug administered or exhibited an inverse dose dependency. To further confirm or disprove true-accompanied and other cholephilic complexes, follow-up studies were conducted that included immunohistochemistry (IHC) assays, transmission electron microscopy (TEM), and/or serum complement activation. The most useful IHC reagents to assess ADA-drug complexes in fixed or frozen monkey tissues included an anti-human IgG reagent to stain for drug as well as reagents for monkey IgG, IgM and complement (C3 and SC5b-9). TEM and compliment activation data added sup-port for verification of involvement of ADA-mediated immune complexes. In to-tality, the data were used to support rationale for clinical dose selection and safety monitoring for the various programs at different stages of development.

BILE SALT TRANSPORT AND LIVER INJURY.

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Bile formation is one of the key functions of mammalian liver. It involves vectorial transport of bile acids across the canalicular membrane, export to the sinusoidal blood into bile. Thereby, bile acids are concentrated more than 500-fold in bile as compared to sinusoidal blood. This concentrative, energy-driven process is dependent on the bile salt export pump (BSEP). Pathophysiological alterations in BSEP function by inherited mutations, inhibition of function by drugs, or disease development can lead to the development of complex liver disease. Drug-induced disruption of BSEP and concomitant bile acid accumulation has been implicated in the development of clinical liver injury for several marketed or withdrawn compounds. Unfortunately, nonclinical models are not reliable predictors of the liver injury associated with BSEP inhibition. However, in vitro screening systems exist to quantitatively assess a compound’s propensity to interfere with BSEP function. In the absence of a relevant preclinical model for BSEP-mediated liver injury, the tox-icolorelevance of available in vitro models to human health require the use of benchmark compounds with known clinical outcomes, such as marketed or with-drawn drugs. This report will describe our efforts to use such compounds in order to better understand the relationship between inhibition of BSEP and the likely-hood of clinical liver injury with the aim of enabling the translation of BSEP inhibi-tion to an approximated in vivo risk through the use of various preclinical in vitro and in vivo models. Various weighting factors, that influence the translation from the in vitro BSEP IC50 to clinical risk of liver injury, are also discussed. Finally, we describe our efforts to identify biomarkers to monitor these effects and the various properties of molecules that may confound the interpretation of these markers.

BILE SALT PUMP (BSEP) REGULATION IN ACQUIRED CHOLESTATIC LIVER DISEASES.

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The bile salt export pump (BSEP, ABCB11) is the rate limiting step in hepatic bile salt excretion and plays as a critical role for hepatic bile salt homeostasis. As such, expression of BSEP is controlled via the nuclear bile salt receptor FXR which senses hepatic, enteric and systemic bile salt concentrations. Genetic BSEP variants can cause a spectrum of cholestatic disorders ranging from progressive familial intra-hepatic cholestasis in neonates and infants to predisposition to intrahepatic cholestasis of pregnancy and drug-induced liver injury in adults. Moreover, various acquired cholestatic injuries such as hepatic and systemic inflammation, drugs (including idiosyncratic reactions associated with considerable hepatic inflammation) and biliary obstruction can interfere with expression and/or function of BSEP and thus contribute to cholestasis. Moreover, BSEP variants could also be involved in the pathogenesis and susceptibility to chronic cholangiopathies such as primary bili-ary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) by contributing to a more toxic biliary milieu. In contrast to its repression in acute (mostly inflamma-tory) cholestatic conditions, BSEP expression is surprisingly well maintained over time and even transiently induced in PBC and PSC, probably as a response to in-creasing bile salt levels activating FXR. Apart from a better understanding of the pathophysiology of cholestasis, these novel molecular insights have also opened new perspectives in diagnosing, predicting and treating BSEP-related cholestatic disor-ders. As such ursodeoxycholic acid (UDCA), the only FDA-approved drug to treat chronic cholestasis, stimulates BSEP expression and function. More novel nuclear receptor (e.g. FXR) ligands can also be expected to target BSEP which may be of in-terest in treating cholestatic disorders associated with loss of BSEP function.

BSEP INHIBITION AS A CONTRIBUTOR TO DRUG-INDUCED LIVER INJURY IN HUMANS.


Drug-induced disruption of BSEP is hypothesized to play a role in the develop-ment of liver injury for several marketed or withdrawn therapeutics. Unfortunately, preclinical animal models, in general, have not been reliable predictors of the liver injury associated with BSEP inhibition. However, in vitro screening systems exist to quantitatively assess a compound’s propensity to interfere with BSEP function. In the absence of a relevant preclinical model for BSEP-mediated liver injury, the tox-icolorelevance of available in vitro models to human health require the use of benchmark compounds with known clinical outcomes, such as marketed or with-drawn drugs. This report will describe our efforts to use such compounds in order to better understand the relationship between inhibition of BSEP and the likely-hood of clinical liver injury with the aim of enabling the translation of BSEP inhibi-tion to an approximated in vivo risk through the use of various preclinical in vitro and in vivo models. Various weighting factors, that influence the translation from the in vitro BSEP IC50 to clinical risk of liver injury, are also discussed. Finally, we describe our efforts to identify biomarkers to monitor these effects and the various properties of molecules that may confound the interpretation of these markers.

IMPAIRED HEPATIC BILE ACID TRANSPORT AND DRUG-INDUCED HEPATOTOXICITY: MECHANISMS AND MODEL SYSTEMS.

K. L. Brunower. School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC. Sponsor: H. Hamadeh.

Accumulation of bile acids in hepatocytes due to inhibition of the canalicular bile salt export pump (BSEP/ABCB11) has been proposed as one mechanism of drug-induced liver injury. Some hepatotoxic compounds also are potent inhibitors of bile acid uptake by Na+-dependent taurocholate co-transporting polypeptide (NTCP/SCL10A1). Accurate predictions of clinically relevant impaired bile acid transport by drugs, and associated hepatotoxicity in patients, is challenging for many reasons including species differences in composition of the bile acid pool, differ-ences in physicochemical potential of various bile acids, species differences in the po-tency of drug-mediated inhibition of hepatic bile acid transport, and the possibility of more potent inhibition by hepatic-derived metabolites than parent drug. Useful in vitro models (e.g., sandwich-cultured rat and human hepatocytes employing a novel cassette dosing approach, suspended hepatocytes, transfected systems) to in-vestigate impaired hepatic bile acid transport and examine mechanisms of drug-in-duced hepatotoxicity will be discussed. Strengths and limitations of each model sys-tem, methods of data analysis, and a discussion of current knowledge gaps and future directions in this clinically important field of research will be discussed. Supported by NIH GM41935.

INHIBITION OF HEPATOBILIARY TRANSPORTERS BY A NOVEL KINASE INHIBITOR CONTRIBUTES TO LIVER TOXICITY IN NONCLINICAL SPECIES.

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Following oral administration of a novel poly cyclic kinase inhibitor, beagle dogs ex-perienced an acute liver toxicity that was characterized by increases in serum bio-markers commonly associated with hepatotoxicity; particularly noteworthy was a
reversible elevation in serum bile salts and total bilirubin. Accompanying this ob-
servation was an ADME appraisal which included hepatic bioactivation and in
vitro inhibition of key hepatobiliary transport proteins. Simply attenuating the
bioactivation proved ineffective in improving the in vivo tolerability of this poly-
cyclic scaffold. Hence, we considered disruption of hepatobiliary transport by the
compound series a likely mechanism contributing to the acute hepatotoxicity.
Indeed, closer in vitro examination employing MDCK cell lines and membrane
vesicles revealed potent, concentration-dependent inhibition of human multi-drug
resistance associated protein (MRP2; IC50 = 30 μM) and bile salt export pump
(BSEP; IC50 = 10 μM), two crucial hepatobiliary transport proteins accountable for
bilirubin and bile salt homeostasis, respectively. Introduction of pKa-altering mod-
ifications to a second generation compound proved successful in reducing its affin-
ity for these key efflux transporters (MRP2 IC50 > 200 μM; BSEP IC50 > 70 μM),
consequently mitigating this overt organ toxicity in nonclinical species. Our results
underscore the importance of transport inhibition screens as a means to predict po-
tential hepatotoxicity inherent to new chemical entities.

626 UNDERSTANDING THE CROSS-TALK BETWEEN BILE SALTS EXPORT PUMP (BSEP) AND OTHER EFFLUX TRANSPORTERS IN THE MANIFESTATION OF DRUG-INDUCED LIVER INJURY.
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Theoretic drugs such as troglitazone, cyclosporine A and bosentan have been im-
plied in idiosyncratic liver toxicity in humans. Common to all of these drugs, is
their ability to interfere with the function of the canalicular transporter bile salt ex-
port pump (Bsep). Bsep inhibition leads to intracellular accumulation of bile acids,
which damage hepatocytes. In addition, Bsep inhibitors may be metabolized to re-
active intermediates that produce oxidative stress. This illustrates the multi-facto-
nal nature of hepatotoxicity produced by these drugs. Bile acid retention and oxy-
idative stress activate compensatory rescue pathways in rodent and human liver.
FXR is a nuclear hormone receptor that functions as a bile acid sensor to coordi-
nately regulate the expression of bile acid-responsive genes. Similarly, oxidative stress activates the Nrf2 transcription factor, which enhances cytoprotective de-
fenses. Other transcription factors, such as CAR and PXR, also mediate critical he-
atobiliary responses to bile acid accumulation. Induction of efflux transporters dur-
ing drug-induced intrahepatic cholestasis is well-documented. Transporters such as
Mdr1, Mrp3 and Mrp4 have been studied as alternative efflux routes for accumu-
lated bile acids when Bsep function is compromised. This presentation will focus on
the current understanding of Mrp4 induction in rodents and patients with drug-induced hepatotoxicity. Emphasis will be placed on the molecular regulation of the ABCG8 (Mrp4) gene and its function in the cholestatic liver and during ox-
idative stress resulting from xenobiotic treatment. An increased knowledge on the
function and regulation of Mrp4 during cholestasis and drug-induced liver disease is
important to understand why a subset of patients develop idiosyncratic liver re-
actions to certain medications.

627 MAP KINASE SIGNALING: A COMMON TARGET IN DIFFERENT TISSUES.
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The mitogen-activated protein kinase (MAPK) signal transduction pathways are
triggered by a variety of extracellular stimuli. Upon activation, MAPKs phosphory-
late downstream targets, transducing these extracellular stimuli into cellular re-
sponses. Since their identification, MAPK signal transduction pathways have been
found to regulate diverse and critical cellular processes such as gene expression, cell
proliferation, differentiation, motility, survival and apoptosis, by altering the phos-
phorylation status of key regulatory proteins. Numerous studies have revealed that
activation of MAPK signaling cascades also occurs in response to a variety of chem-
ical and physical stresses. The activation status of critical MAPKs, including extra-
cellular signal-related kinase (ERK), p38 and c-Jun N-terminal kinase (JNK), may
be modulated by exposure to xenobiotics. Ongoing research continues to elucidate
the role of MAPK signaling alterations during chemical-induced toxicity. Despite
the ubiquitous nature of MAPK signal transduction pathways, the modulation and
function of each individual MAPK has been suggested to be highly cell type and con-
text dependent. Therefore, in-depth studies are needed to understand the mech-
anisms underlying tissue-specific toxicity involving alterations of MAPK signaling
pathways. This session will highlight the most recent research progress made to
characterize the alterations of MAPK signaling pathways in response to toxicant ex-
posures, and how these alterations contribute to toxicity and/or pathogenesis in dif-
ferent tissues and cell types. The qualitative comparison among data presented in
this session will either suggest a paradigm of MAPK response to various toxicants,
or illustrate the cell type/tissue specific difference in the role of MAPK signaling al-
terations during toxic responses.

628 GENE EXPRESSION STUDIES DEMONSTRATE THAT THE K-RAS/ERK MAP KINASE SIGNAL TRANSDUCTION PATHWAY CONTRIBUTES TO THE PATHOGENESIS OF CUMENE-INDUCED LUNG TUMORS.
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Lung cancer is the most frequently diagnosed cancer in the world and the most
common cause of cancer mortality worldwide. Mouse models have been used to
study carcinogenesis of human lung cancers and many of the major genetic alter-
ations detected in humans have also been identified in mice. Cumene or isopropyl-
benzene is a component of crude oil used primarily in the production of phenol and
eacetone. The National Toxicology Program demonstrated that exposure to
cumene significantly increased the incidence of alveolar/bronchial adenomas and
carcinomas in B6C3F1 mice. Cumene-induced lung neoplasms in mice were eval-
uated for point mutations in the K-ras gene using cycle sequencing of PCR-ampli-
fied DNA from paraffin-embedded neoplasms. K-ras mutations were detected in
87% of cumene-induced lung neoplasms and the predominant mutations were
exon 1 codon 12 G to T transversions and exon 2 codon 61 A to G transitions.
Global gene expression analysis was performed to distinguish patterns of gene regu-
lation between cumene-induced lung tumors and normal lung tissue and to look
for patterns based on the presence or absence of K-ras mutations in the tumors.
Principal component analysis segregated the carcinomas into groups with and
without K-ras mutations. Expression of genes associated with the Erk MAP kinase sig-
naling pathway was significantly altered in carcinomas with K-ras mutations com-
pared to tumors without K-ras mutations or normal lung. Gene expression analysis
also suggested that cumene-induced carcinomas with K-ras mutations have greater
malignant potential than those without mutations. Our gene expression analysis
suggested the formation of alveolar/bronchial carcinomas in cumene-exposed
mice typically involves mutation of K-ras, which results in increased Erk MAP ki-
nae signaling.

629 ROLE OF MAP KINASES AND PHOSPHATIDYLINOSITOL-3 KINASE/AKT IN REGULATING KERATINOCYTE ANTIOXIDANT EXPRESSION IN RESPONSE TO 4-HYDROXYNENAL, A LIPID PeroxidATION END PRODUCT.
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Piscataway, NJ, 2Environmental Health, New York Medical College, Valhalla, NY
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4-Hydroxynonenal (4-HNE), an electrophilic lipid peroxidation end product, is
generated in cells following oxidative stress in response to a variety of agents in-
cluding chemical vesicants and UVB light. As a highly reactive aldehyde, 4-HNE
readily forms adducts with macromolecules including proteins, lipids and DNA. In
the skin, keratinocytes contain a diverse array of antioxidant and redox proteins that
protect cells against oxidative stress. We used primary cultures of mouse ker-
atinocytes and PAM 212 keratinocytes to determine if 4-HNE modified ker-
atocytic expression of cellular antioxidants. 4-HNE was found to induce mRNA
expression of heme oxygenase-1 (HO-1), glutathione-S-transferase A1-2 (GSTA1-
2) and NAPDH: quinone oxidoreductase (NQO-1) in both cell types. HO-1 and
Gsta1-2 were most responsive to 4-HNE, maximal increases of HO-1 (86-97 fold)
were evident in cells treated with 30 μM 4-HNE for 6 hr while increases in
Gsta1-2 mRNA (30-63 fold) were maximal after 24 hr. Using PAM212 cells, we
found that 4-HNE (30 μM) activated both the JNK and p38 MAP kinase and
phosphatidylinositol (PI)-3 (PI3K/Akt) signaling pathways while ERK1/2 MAP ki-
nae was constitutively activated. Inhibition of the PI3/Akt, JNK, ERK1/2 and p38
MAP kinases markedly suppressed 4-HNE-induced expression of HO-1 and
NQO-1, but not GSTA1-2. Taken together, these data indicate that 4-HNE modu-
lates expression of antioxidant enzymes in keratinocytes by distinct signaling path-
ways.
Parkinson’s disease (PD) is characterized by selective loss of dopaminergic neurons in the substantia nigra of the brain. Although the underlying causes are not well characterized, epidemiological studies suggest an elevated risk of PD with occupational pesticide exposure. Here, we utilized PC12 and SH-SYSY cells as well as rat primary cultured dopaminergic neurons to investigate mechanisms of dopaminergic cell death induced by paraquat and rotenone, pesticides that are used to model PD in rodents. Both paraquat and rotenone induce selective loss of dopaminergic neurons in primary cultures. We discovered that paraquat induces apoptosis in PC12 cells but not in SH-SYSY cells, while rotenone exposure causes apoptosis in SH-SYSY but not PC12 cells. In addition, we report that rotenone-induced apoptosis in SH-SYSY cells is attenuated by pretreatment with basic fibroblast growth factor (bFGF). bFGF activated both extracellular signal-regulated kinase 1/2 (ERK1/2) and phosphatidylinositol-3 kinase (PI3-kinase) pathways in SH-SYSY cells. The selective ability of paraquat and rotenone to induce apoptosis in different cell lines correlates with their ability to activate c-Jun N-terminal protein kinase (JNK) and p38 mitogen-activated protein (MAP) kinases. Furthermore, both JNK and p38 are required for rotenone-induced apoptosis in SH-SYSY cells as well as primary neurons, and for paraquat-induced apoptosis in PC12 cells. However, JNK but not p38 plays a role in paraquat-induced death of primary cultured dopaminergic neurons. Our data identify JNK activation as a common mechanism underlying dopaminergic cell death induced by both paraquat and rotenone in model cell lines and in primary cultures.

Some chemicals are known to suppress innate immunity and inflammation, such as ethanol (EtOH) and Sodium methyldithiocarbamate (SMD), an abundantly used soil fumigant. Our lab demonstrated that both EtOH and SMD suppress inflammation in primary neurons, and for paraquat-induced apoptosis in PC12 cells. However, JNK but not p38 plays a role in paraquat-induced death of primary cultured dopaminergic neurons. The selective ability of paraquat and rotenone to induce apoptosis in different cell lines correlates with their ability to activate c-Jun N-terminal protein kinase (JNK) and p38 mitogen-activated protein (MAP) kinases. Furthermore, both JNK and p38 are required for rotenone-induced apoptosis in SH-SYSY cells as well as primary neurons, and paraquat-induced apoptosis in PC12 cells. However, JNK but not p38 plays a role in paraquat-induced death of primary cultured dopaminergic neurons. Our data identify JNK activation as a common mechanism underlying dopaminergic cell death induced by both paraquat and rotenone in model cell lines and in primary cultures.

Decades of research have established 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) as a potent suppressor of the immune response, particularly for the IgM antibody response. Previous studies have shown the B cell is highly, and directly, sensitive to TCDD. Exposure to TCDD results in a failure of activated B cells to differentiate into antibody-secreting cells. Results obtained in vivo suggest that TCDD prevents expression of the transcription factor Blimp-1, a central regulator of B cell to plasma cell differentiation. Blimp-1 expression is indirectly controlled by multiple kinases, including AKT, ERK, and JNK. It was hypothesized that TCDD prevents Blimp-1 induction by altering phosphorylation of AKT, ERK, and JNK. Murine thymic B cells were treated by simultaneous addition of Toll-like Receptor (TLR) ligand and TCDD (0.003, 0.03, or 0.3 nM TCDD), then kinase phosphorylation status was fixed by direct addition of formaldehyde at 15, 30, or 60 min post-treatment. Phosphorylation status of AKT, ERK, and JNK was determined by multiparametric flow cytometry. LPS, CpG, and R848 all activated kinase phosphorylation to varying degrees, with LPS the least potent and R848 the most potent activators of kinase phosphorylation. CpG caused the most phosphorylation of all kinases examined. Immunosuppressive concentrations of TCDD, 0.03 and 0.3 nM, significantly impaired TLR-activated AKT, ERK, and JNK phosphorylation at all time points. TCDD at 30 nM also significantly impaired R848-activated AKT, ERK, and JNK phosphorylation in primary mouse B cells. Collectively, these data provide a partial explanation for the mechanism of TCDD-mediated suppression of the TLR-activated primary IgM response.

Metabolic disorders have gained increasing recognition as important outcomes to many toxicities, the etiologies of which include environmental and occupational exposures, as well as adverse drug reactions. Because of its fundamental role in cell bioenergetics and intermediary metabolism, the mitochondrion is implicated in the pathogenesis of many of these disease states. While the majority of studies have been directed at understanding the acute response to mitochondrial toxicity, only recently have investigators come to realize the significance of subtle molecular changes that occur in response to mitochondrial injuries that define the metabolically compensated state of the cell. Such changes are essential to the cell being able to withstand low doses and chronic exposures to agents that interfere with mitochondrial function. Collectively, it is these events that define the biological response to sub-lethal exposures and that offer unique opportunities for identifying exposures that may otherwise go unrecognized as a potential metabolic liability for the individual. To adequately address these issues, a general overview of mitochondrial metabolism followed by a series of focused discussions of the molecular changes that define the biological response will be highlighted. The session concludes by leveraging this understanding of the molecular response to the identification of potential biomarkers for reporting subtle, non-clinical cases of mitochondrial toxicity.
bioenergetics. In this presentation, I will describe our work with adriamycin-in-
duced mitochondrial dysfunction and cardiac toxicity, and the altered metabolome
that we propose is responsible for sustaining cardiac performance in the face of
overt mitochondrial toxicity. The critical question is the identity of the bioenergetic
signals and molecular sensors that control this compensated metabolic state essen-
tial to survival in mitochondrial disease.

**TRANSCRIPTIONAL PROFILING OF MITOCHONDRIAL TOXICITY.**

V. G. Desai and J. C. Fuscoe, Division of Systems Toxicology, NCTR, U.S. FDA,
Jefferson, AR.

A growing body of evidence indicates significant involvement of mitochondria in
various drug-related toxicities and disease processes; however, the underlying mech-
anisms are still poorly understood. Mitochondria are complex cellular organelles
that possess intricate networking of various molecular pathways. Understanding of
the mitochondrial function in drug toxicities and diseases, therefore, requires a
comprehensive approach for examination of the many aspects of mitochondrial
function. A mitochondria-specific mouse oligonucleotide microarray (Mitochip)
was developed for the molecular profiling of the expression of 542 genes involved
in mitochondrial structure and function. In the first validation experiment, this ge-
nomic tool was utilized to delineate the mechanistic basis of impaired mitochondr-
ion function in different mouse models exposed to nucleoside reverse transcriptase
inhibitors (NRTIs) used in the treatment of HIV-1 infections. Data revealed that
NRTI-mediated altered mitochondrial activity may be multi-factorial and that
mechanisms responsible for mitochondrial dysfunction in the liver and skeletal
muscle may be different. The Mitochip was also used in understanding the role of
mitochondria in hepatotoxicity caused by the weight-loss supplement eutnic acid in
a mouse model. Transcriptional profiling of mitochondria-related genes in the liver
showed alterations involving oxidative phosphorylation and its interaction with
other critical mitochondrial pathways at the genomic level. Collectively, data from
these studies show the utility of the Mitochip as a valuable genomic tool to deter-
mine the mechanisms of drug toxicities or degenerative diseases associated with al-
tered mitochondrial function.

**METABONOMIC AND FLUXOMIC FINGERPRINTING OF METABOLIC AND MITOCHONDRIAL STRESS.**


The ultimate response of biological systems to chemical, physical, and environmen-
tal stress is reflected on metabolite and signaling molecule levels. Evaluation of
metabotype of organisms and stress response requires knowledge not only metabo-
lite levels but also their turnover rates from which metabolic fluxes and status of
metabolic system can be determined. Changes in metabolite dynamics or fluxomics
are more sensitive indicators of any mitochondrial and metabolic perturbations.
Developed at the Mayo Clinic oxygen-18-assisted 31P NMR and mass spectromet-
ric technique allows simultaneous measurements of phosphorus-containing
metabolites in tissues and body fluids to establish a metabonomic and fluxomic fin-
gerprint useful in the differential diagnosis of the degree of mitochondrial stress. In
targeted phosphometabonomic analysis up to 100-150 phosphorus-containing
metabolites and turnover rates can be quantified using 31P NMR spectroscopy,
LC/MS and GC/MS with prior HPLC separation of metabolites classes. This tech-
nology permits simultaneous recordings of ATP synthesis and utilization, phospho-
transfer fluxes through adenyate kinase, creatine kinase and glycolytic pathways
as well as mitochondrial Krebs cycle activity and glycolgen turnover. Another advan-
tage of 18O methodology is that it can measure almost every phosphotransfer reac-
tion taking place in the cell including turnovers of important signaling molecules
such as cAMP, cGMP and AMP and their metabolically active pool sizes. Our stud-
ies demonstrate that this approach is valuable tool for metabolic and fluxomic
profiling of transgenic animal models simulating human diseases, hypoxic response
as well as preconditioned and failing hearts.

**MITOCHONDRIAL BIOGENESIS – RESCUE FROM METABOLIC DISORDERS.**

R. G. Schnellmann, Pharmaceutical Sciences, Medical University of South Carolina,
Charleston, SC.

Dr. Schnellmann will review the current understanding of key signaling molecules
and pathways intimately involved in stimulating mitochondrial biogenesis and
their role as part of the compensatory response to mitochondrial toxicity. He will
then describe recent research using new molecules developed in his lab that are ef-
fective in restoring mitochondrial function by stimulating the mitochondrial bio-
genesis pathway. Such molecules afford important opportunity in the clinical man-
agement of mitochondrial dysfunction.

**POPS: WHAT’S NEW AND WHY SHOULD WE CARE?**

A. Schecter1 and L. Birnbaum1, 2. University of Texas School of Public Health at
Dallas, TX and 3. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The persistent organic pollutants, or POPs, are of increasing concern among the
general public, health care providers, and scientists. These synthetic pollutants are
characterized as being very persistent in biota and the environment, toxic, under-
going trans-boundary migration, and are bioaccumulative. Legacy or classical POPs in-
clude PCBs, organochlorine pesticides, and chlorinated dioxins/furans. Presently,
considerable interest is focused on newly emerging POPs such as brominated flame
retardants (BFRs), including polybrominated diphenyl ethers or PBDEs, and poly-
fluoroalkyl chemicals (PFCS) which include PFOS and PFOA. Tremendous im-
provements in analytical chemistry have improved the rates of detection of these
compounds in the environment and in humans. Increasing levels of many of the
emerging POPs measured in human and environmental samples has become cause
for concern. Questions regarding these compounds include exposure assessment,
toxicity, possible substitutes, interactions, metabolism, and regulations. Therefore,
this session will provide a current overview from leaders in these important areas
and will be followed by detailed recent findings from the scientists actively re-
searching these compounds.

**LEGACY AND EMERGING POPs.**

L. Birnbaum, National Institute of Environmental Health Sciences, Research Triangle
Park, NC.

Legacy POPs include organochlorine pesticides, chlorinated and brominated diox-
ins, and PCBs. In general, their production and use has either been phased out or
severely restricted. Exposure to these chemicals is primarily through contamination
of the food supply because of the persistent nature of these compounds, and their
bioaccumulation and biomagnification up the food chain. They are primarily
found in foods of animal origin including meat, fish, dairy and their byproducts.
However, the emerging POPs of concern involve several classes of brominated
flame retardants and perfluorinated chemicals (PFCs) such as the long chain poly-
fluorinated carboxyl or sulfonic acids, exemplified by PFOS and PFOA. They can all be
found in many consumer products, which can lead to exposure via in-
halation and ingestion of dust from homes and workplaces sometimes as substantial
exposures. In addition, with lower brominated PBDEs are highly lipophilic and
partition into fat tissue, the highly brominated congeners, such as the nona-
and deca-BDEs, tend to be found in blood or liver, rather than in adipose tissue or
lipid. This is also true of the PFCs which also fail to partition into fat. Thus, expo-
sure to these newer POPs involves other exposure sources than primarily fatty foods
of animal origin. This abstract does not reflect NIEHS/NIH policy.

**ARE CONCENTRATIONS OF POLYFLUOROALKYL CHEMICALS IN THE GENERAL U.S. POPULATION ARE DECLINING?: DATA FROM THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEYS (NHANES).**

A. Calafat, L. Wong, K. Kato, Z. Kuklenyik and L. L. Needham, Center for Disease Control and Prevention, Atlanta, GA.

We have measured the serum concentrations of various polyfluoroalkyl chemicals
(PFCS) in participants of several U.S. National Health and Nutrition Examination
Surveys (NHANES) both before and after the phase-out manufacture by electro-
We will discuss the usefulness of biomonitoring programs, using NHANES as an
example, to provide evidence of exposure and absorption of PFCS in humans and to
assess temporal changes in internal dose when actions are taken that lead to changes
in the environmental concentrations of these chemicals.
We previously reported high levels of PBDEs in the U.S. population and their increase during the past few decades, along with a decline in dioxins, dibenzofurans and PCBs. We found high levels of PBDEs in market basket surveys of U.S. food but not high enough to account for the elevated levels in humans vis-à-vis European levels. Differing sharply from PBDEs, hexabromocyclododecane (HBCD) levels in human milk in the U.S. are not higher than those in fish. PBDE levels in ongoing food surveys varied with fish usually higher but US consumption calculations estimated higher meat intake. We found no geographical differences in PBDE levels in food from California, New York, and Texas supermarkets. We concluded that dust appeared to contribute to PBDE body burden in humans in the U.S. Foods of animal origin were usually higher in PBDEs than vegetables and fruit. Our current food studies measure PBDEs, PFCs, marker PCBs, pesticides and HBCD, from pooled samples. This new study extends our findings to include a variety of POPs, including those not previously reported in U.S. food. Measured levels of certain classical and emerging POP congeners were found in each food type analyzed. Clear patterns within food types were not always seen. However, sardines and salmon usually had elevated PBDE levels. Butter was found to have very elevated PBDE levels, primarily from BDE 209.

Concentrations of several historic POPs, i.e. polychlorinated dibeno p dioxins, polychlorinated dibenzo furans, and polychlorinated biphenyls, along with a newer class of POPs, the polybrominated diphenyl ethers (PBDEs), were measured in domestic meat and poultry samples from 2002 and 2008. The meat and poultry samples included beef, market hogs, young chickens, and young turkeys and were collected as part of a statistically-based survey of dioxins by the U.S. Department of Agriculture. Comparison of the data from the two collection periods shows a declining trend for each of these pollutant classes. The median concentration of dioxin-like compounds decreased 6 – 25% in beef, chicken, and turkey; pork levels showed no change but remained at the lowest level. For PBDEs removed from production in the U.S. in 2004, the mean concentrations decreased 50 – 80% in each production class. These declining trends in food illustrate the effectiveness of regulations and surveillance programs and may result in corresponding declines in human levels.

Studies in mammals have shown that lipophilic tissues such as adipose and skin are the major reservoirs for polybrominated diphenyl ethers (PBDEs). Because humans commonly consume the skin of chicken, it was of interest to study the metabolic behavior of the most abundant PBDE found in biota, i.e. 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in this production avian species. Single-oral-dose results demonstrated that, as previously shown in rats and mice, BDE-47 was well absorbed from the gut (nearly 61% remained in tissues at 72h), and was distributed both on a %dose basis and a concentration basis to lipophilic tissues, i.e. adipose tissue, skin and GI tract. BDE-47 levels in these tissues were 3-35 times greater than in the edible muscle, and dark meat contained three times more BDE-47 than white meat. Metabolism to free metabolites was less than 1% of the dose, although non-extractables in the excreta amounted to 12% of the dose. Excreted metabolites had undergone a variety of oxidative and debrumination events. These results demonstrated that edible chicken tissues may serve as a good route of PBDEs in humans, however, trimming fat and skin may dramatically reduce these exposures.

The two-year rodent bioassay is currently the most expensive and time-consuming animal test required for pharmaceutical and chemical carcinogenicity assessment. A vision for the 21st century is proposed for a staged approach to altering the current pharmaceutical carcinogenicity testing paradigm that reduces the timeline, animal and human resources, and improves human risk assessment. Analyses of decades of shared data from pharmaceutical carcinogenicity testing are helping to define an approach to preserve protections and benefits afforded to patients, while providing support for a near-term significant modification to testing guidelines. These data are also helping to define a research strategy that will deliver further improved testing. In the past decade, genetically modified animal models have been introduced and incorporated as an additional to the pharmaceutical test battery option. A new paradigm supported by decades of test data proposes to maximize the value of such mouse models to minimize the need for two-year rat carcinogenicity studies. Furthermore, advances are being made through collaborative research initiatives that point to anticipated growth in qualified biomarkers for monitoring in both animal models, and in vitro test systems that may allow quicker resource sparing approaches to improved cancer risk identification. These approaches promise not only to allow earlier identification of rodent tumorigenic chemicals, but also to provide deeper insights into mode-of-action and better understanding of human relevance. Therefore it is a goal of this session to provide new understanding based on the key lessons learned from decades of pharmaceutical testing experience, of emerging carcinogenic based biomarkers, of development of in vitro screening and mechanistic models, of integrated mode-of-action systems biology based approaches to identify and assess genotoxic and nongenotoxic mechanisms, and offer regulatory perspectives on the impact of these developments on the near-term and long-term future of carcinogenicity testing.
proposed testing paradigm yielded 80% test sensitivity and negative predictivity for rat carcinogenicity outcome. Of the 14 false negative compounds found among the 180 compounds studied, their development was not discontinued because of the rat carcinogenicity outcome, and the compounds were mainly single sex, single species, or single site carcinogens of questionable human relevance. The results indicate little difference between 6 and 12-month chronic toxicology studies for predicting 2-year outcome. Such data may prove useful as a basis for revising international guidelines relating to requirements for rat carcinogenicity testing while preserving and potentially improving upon the protections and benefits afforded to patients. When considered together with a paradigm incorporating the 6-month transgenic mouse tumorigenicity study, these data may support significant reductions in the timeline to market for important pharmaceuticals critical to patients in need, and contribute significantly to sensible reductions in animal use. Furthermore, a deeper analysis of patterns in the data is providing the basis for a testing strategy to launch qualification studies of proposed new molecular biomarkers and model systems for earlier prediction of chemical tumorigenic risk.

**647 A PROPOSED VISION ON THE FUTURE OF CARCINOGENICITY TESTING.**

D. Jacobson-Kram, CDER/U.S. FDA, Silver Spring, MD.

The FDA Center for Drug Evaluation and Research has demonstrated an eagerness to adopt new approaches to toxicology testing that preserve patient protections and improve delivery of safe products to patients. The 2-year carcinogenicity testing paradigm defined since the 1960s and 1970s has been a key component since the Food, Drug, and Cosmetic Act of 1938 currently in place for accelerating patient accessibility to new products. The FDA has had years of experience now with transgenic mouse models to support product development and has developed SAR modules for prediction of carcinogen potential. Perspectives on these as well as concrete steps that can be taken in the near term as well as the research strategy for the long term modification of ICH harmonized approaches to regulatory testing requirements will be discussed.

**648 INTEGRATING PREDICTIVE AND MECHANISTIC CARCINOGENICITY BIOMARKER INTO DRUG DISCOVERY AND DEVELOPMENT.**

M. Fielden, Amgen, South San Francisco, CA.

Carcinogenicity testing has evolved slowly since the two-year rodent bioassay became the standard means of evaluating the carcinogenic risk of new chemical entities. Despite our modern genomic tools, we are still challenged in converting our knowledge and capabilities into tangible benefits for the early prediction and risk assessment of non-genotoxic carcinogens. While in vitro and in vivo genomics have been touted as promising tools to aid toxicity prediction, their effectiveness may be realized only when integrated with more traditional endpoints and approaches to form the basis of an improved human risk assessment and to achieve gains in non-clinical testing efficiencies. Collaboratively, the Predictive Safety Testing Consortium of the Critical Path Institute aims to facilitate the early risk assessment of new chemical entities by advancing the ability to predict and to understand mechanistically rodent non-genotoxic hepatocarcinogenesis. Through retrospective evaluation of published work and cross-laboratory biomarker evaluation, we identified a robust set of 23 genes for the classification and mechanistic understanding of genotoxic and non-genotoxic hepatocarcinogenesis. Transferring these biomarker genes onto the Taqman® Low Density Array facilitated the re-derivation and validation of a hepatic gene expression-based signature for the prediction of non-genotoxic hepatocarcinogenesis.

**649 IMPROVING CANCER RISK ASSESSMENT OF DRUG CANDIDATES BY INTEGRATING SYSTEMS BIOLOGY TO DEFINE MODE-OF-ACTION OF CARCINOGENS.**

J. Aubrecht, Drug Safety Research and Development, Pfizer Inc., Groton, CT.

Despite the scientific progress in understanding of carcinogenicity, experimental approaches for assessing oncogenic risk associated with exposure to chemicals relies mainly on methods originally developed in 1960- and 1970-ties. In many instances, the limited mechanistic insights provided by currently used approaches do not provide sufficient information to assess oncogenic risk to humans. Recent advances in molecular biology and bioinformatics have enabled interrogating cellular responses to chemical exposure on genomic level eventually leading to identifying molecular pathways and networks mechanistically involved in chemical carcinogen- esis (system biology approach). Although systems approaches provide valuable biological insights, new experimental approaches are necessary to understand cross species differences in molecular pathways ultimately leading to better cancer risk as- sessment for humans. Along these lines, recent progress in the development of “hu- manized” experimental models provides unique opportunities to complement pathway based approaches. These models include transgenic animals and animals bearing human tissues. This presentation will summarize the current state of applying integrated systems biology approaches to understand carcinogenic modes/mechanism of action and in conjunction with humanized models provide a case study for facilitating human cancer risk assessment of drug candidates.

**650 USING IN VITRO HAZARD IDENTIFICATION APPROACHES FOR IMPROVING HUMAN CANCER RISK ASSESSMENT AND PRECLINICAL TESTING.**

J. H. van Delh1,2, C. Magkouloupolou1,2, K. Mathijs1,2, D. G. Jennen1,2, D. Lizarraga1,2, S. H. Claessen1, K. J. Braeusers1 and J. C. Kleijmans1,2.

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The high false positive rate of conventional in vitro genotoxicity assays and the lack of in vitro models to screen compounds for non-genotoxic carcinogenic properties, raise the need for development of alternative in vitro methods. Toxicogenomic approaches are thought to be able to contribute to this need. In our research program, the major aim is to develop mechanism-based in vitro methods for assessing the genotoxic and carcinogenic potential of compounds, as an alternative to current rodent bioassays. As target organ the liver is incorporated, with some efforts on lung and kidney. Most novel assays are based on the application of transcriptionomics of mRNA and miRNA, and to some extent also metabonomics. Some assays include robust in vitro systems including differentiated human stem cells, in order to generate “omic” responses using a well-defined set of model compounds. Through extensive use of biostatistics, differential genetic pathways may be identi- fied as a tool for identifying mechanisms of chemical carcinogenesis in vivo. In both HepG2 cells and sandwich-cultured mouse primary hepatocytes, discrimination of true genotoxic from false-positive genotoxic compounds was achieved with ~80% accuracy. Optimal treatment periods were very different between both models, being 12-24h in HepG2 cells and 48h in primary hepatocytes. Gene classifiers were also mostly different, though in both models genes related to apoptosis were af- fected. Applying pathway expression changes generated by ToxProfiler instead of gene expression changes, did not improve the prediction. We conclude that pathway analysis is promising as a tool to identify mechanisms induced by toxigenomics technologies. This research is sponsored by the European Union project carcinogenomics and by several Netherlands organisations.

**651 RESEARCH ADVANCES AND ENDURING NEEDS IN CHILDREN’S ENVIRONMENTAL HEALTH PROTECTION.**

S. P. Darnay, ORD, U.S. EPA, Research Triangle Park, NC.

Children may be more vulnerable and susceptible to health impacts of environ- mental contaminants based upon age-specific behaviors that increase exposure; de- velopmental processes that are differentially susceptible to disruption; and genetic variables that alter biological responses to toxicants. Federal laws require considera- tion of children’s unique vulnerability in rule making activities. Recent research about how children are exposed and react to environmental contaminants helps de- cision makers set environmental standards based on scientific information rather than current assumptions. In 1998, the U.S. EPA and NIEHS established the Children’s Environmental Health and Disease Prevention Research Program. These centers are examining interactions between key environmental exposures and a range of child prevalent diseases such as asthma and autism. Emphasizing multidisciplinary basic, applied, and community-based participatory approaches, their common goal is to reduce children’s health risks from environ- mental contaminants, prevent childhood diseases, and share findings with the af- fected communities and the broader public. In 2009, NIH launched the National Children’s Study (NCS) in partnership with CDC, U.S. EPA, and NIEHS. NCS will recruit and follow 100,000 children from before birth to adulthood, gathering both exposure and health outcome information and evaluating how early life expos- ures may affect their subsequent health. Therefore it is important that we seize the opportunity through these initiatives to synthesize recent research advances in child-specific exposure science and health effects, including lessons learned from the Children’s Centers, illustrate how these advances are being integrated into new
studies such as the NCS, consider how this new knowledge can be used in risk assessment, and address ongoing challenges. Specifically, these challenges include interpreting biomonitoring data, finding and eliminating exposure sources, predicting health effects, communicating the findings to regulators and the public in meaningful ways, and informing policy decisions.

**652 FEDERAL EFFORTS TO ADDRESS CHILDREN’S ENVIRONMENTAL HEALTH.**

A. Dearth. NIEHS, NIH, Research Triangle Park, NC. Sponsor: S. Darney.

Children face disproportionate and unique threats from environmental hazards for many reasons. Protecting children’s health from environmental pollutants has been a major concern for both EPA and NIEHS. This introduction will focus on work carried out by the EPA-NIEHS Centers for Children’s Environmental Health and Disease Prevention Research, also known as the Children’s Environmental Health Centers (CEHCs), and the newly launched National Children’s Study (NCS). In 1998, NIEHS and EPA initiated CEHCs, which examine the interaction between key environmental exposures and a range of child health outcomes, including overall growth and development, asthma and respiratory health, and neurodevelopmental disorders such as autism. Collectively, these Centers comprise a national network of scientific and community leaders, health care providers and government officials — with the common goals of preventing and reducing childhood diseases in the research areas under study and translating the findings to affected communities and the broader public. NIEHS and EPA have also partnered with NICHD and CDC in development and planning of the NCS since the Children’s Health Act was enacted in 2000. After eight years of such planning, NCS began enrollment and data collection at the first of the initial, or Vanguard, Locations, in Duplin County, NC and Queens, NY in January 2009. The remaining five Vanguard Locations joined them in April 2009, to be followed by 29 additional Locations in 2010 and more in the following two years. With broadly defined environmental exposures and all of children’s health and developmental outcomes of concern, the breadth of the NCS calls for equally broad input from exposure science experts, communities, and involved groups. Findings from such efforts, supported by NIEHS, EPA, NICHD, CDC, and other federal agencies, will ultimately benefit all Americans by providing researchers, health care providers, risk assessors, and public health officials with information from which to develop prevention strategies, health and safety guidelines, and possibly new treatments and cures for disease.

**653 CHILDREN’S EXPOSURES TO CHEMICALS.**


Since the mid-90s, EPA mandated that children’s aggregate exposures to multiple chemicals must be explicitly considered in risk assessment. In response, our exposure research moved to understand where, when, and how children are exposed to chemicals and how differential exposures and susceptibilities impact risks. Exposure research was designed to understand: product use patterns; spatial/temporal variability in contaminant levels; activity patterns and routes of exposure; biomarker/exposure estimates, and exposure/dose modeling tools. Methods for measuring children’s exposures were developed and applied in studies to understand the key processes that impact exposure and to generate quality data. Many chemicals were frequently found in air, dust, food, and wipe samples from settings where children spend time. These chemicals were associated with consumer products (pesticides, cleansers), furnishings (PFCs, PBDEs, phthalates), and combustion processes (PAHs). Pesticide products were found in -90% of homes, with higher use rates in warmer areas. Research showed volatile OP pesticides peak -24h after use and then rapidly dissipate, The less volatile pyrethroids emit slowly with higher use rates in warmer areas. Research showed volatile OP pesticides peak -24h after use and then rapidly dissipate, The less volatile pyrethroids emit slowly with levels likely to increase with repeated use. OCP and OP pesticides are still routinely detected indoors. Dominant exposure routes vary, dietary ingestion dominates for many chemicals, while nondietary ingestion or dermal absorption is primary for pyrethroids. Ongoing research is linking models to simulate exposure and dose, predict biomarker levels, and support cumulative risk assessments. EPA’s exposure research provides important science (e.g., pesticides sources, co-occurrence, form, exposure route) to inform toxicity testing and epidemiological studies. Linked models provide data for health effects researchers to simultaneously estimate exposure and target tissues dose characteristics for multiple pathways and chemicals, and use biomarker data for risk assessments. Exposure science products are directly used in exposure assessments, risk reduction strategies, and educational materials. Although this work was reviewed by EPA, it may not necessarily reflect official Agency policy.

**654 GENE-ENVIRONMENT INTERACTIONS AND CHILDREN’S SUSCEPTIBILITY.**

F. M. Faustman. Center for Child Environmental Health Risk Research, University of Washington, Seattle, WA.

Understanding gene-environment interactions, which define our susceptibility to environmental agents, requires characterization of both exposures to such agents and the genetics that determine our responses to them. The National Children’s Study (NCS) longitudinal cohort provides a unique opportunity for us to better elucidate these interactions related to children’s health. To optimize this opportunity we can build upon a series of studies from the NIEHS and EPA supported Children’s Health Centers (CHC) and we can translate these lessons learned to inform the NCS design and implementation. In so doing, we need to use a life-stage specific framework. This talk will explain three key aspects of this framework, giving examples from the CHC and broader children’s literature for illustration. The talk will end by illustrating how these aspects directly affect how we designed or will modify the design of the NCS following the Vanguard pilot phase and how we are able to translate information from this cohort to answer NCS hypotheses and ultimately inform risk assessment and public health. A seminal lesson learned from the gene-environmental studies from CHC has been the need to consider the dynamics of the gene-environment interaction. Although frequently we consider genetics to be “stable”, many recent findings illustrate that it is important to consider the temporal onset of genetic differences for translating information for the identification of “at risk” populations. For example, studies on the temporal onset of cytochrome P450 and other metabolizing enzymes (such as PON-1) expand our understanding of when adult levels of enzymes and enzymatic differences occur. This timing varies for different forms of the enzymes as well as for the genetic background of the fetus and its mother. Therefore strategic collection of biospecimens that allow for both geno-phenotyping and for understanding the temporal onset of such differences is essential in order to interpret gene-environmental information for children. Supported by EPA RD83273501, NIEHS 5P01ES096901, EPA RD83170921 and NIEHS P30 ES07093.

**655 UNIQUE OPPORTUNITIES IN THE NATIONAL CHILDREN’S STUDY.**

J. Park1, E. B. Clark2, L. E. Palmer2, J. Gilliland2, S. Firth2, P. Silberman2 and J. Johnson2. 1National Children’s Study, Eunice Kennedy Shriver National Institute of Child Health, Bethesda, MD and 2Pediatrics, University of Utah, Salt Lake City, UT. Sponsor: S. Perreault Darney.

The National Children’s Study (NCS) provides unique opportunities to study a host of fetal or early life chemical and environmental exposures and their associations with outcomes in various stages of childhood and adolescence. The NCS cohort of 100,000 children selected from 105 sites will be nationally representative and will allow researchers to confirm exposure-outcome associations reported in smaller, underpowered studies. The NCS’s core hypotheses explore environmental exposures as risk factors for asthma, impaired cognition, diabetes, and reproductive development. The NCS will sample air, house dust, water, soil, and food during the mother’s pregnancy and the child’s early life, and children will be followed prospectively for health effects. The NCS will obtain biospecimens such as urine, saliva, vaginal fluids, blood, breast milk, meconium, and cord blood to analyze for biomarkers of exposures and biomarkers of effects. Contaminants of concern include heavy metals, volatile organic compounds, carbamyls, semi-volatile organic compounds, pyrethroids, disinfection byproducts (heronically active agents), dioxin, polychlorinated biphenyls, pesticides, and persistent organic pollutants. Multiple exposures will be assessed allowing exploration of exposure outcome-relationships where multiple compounds are suspected to interact or to be confounders. For the more pervasive and common exposures and outcomes, statistical models will be able to estimate the proportion or risk attributable to these compounds and, potentially, to their interactions. Because of its large sample size, prospective design, and comprehensive exposure assessment, the NCS will be able to quantify exposure-disease relationships in children more so than any other study heretofore undertaken in the U.S.

**656 HOW CAN SCIENCE INFORM RISK-BASED DECISIONS AND PROTECT CHILDREN’S HEALTH?**


Vulnerability to environmental chemical exposure is determined by a multitude of factors. Life stage and genetic background are both examples of intrinsic factors that determine vulnerability to environmental exposures. Extrinsic factors also have a significant impact on vulnerability. For example, the level of exposure to chemical
stressors can be modified by behavior and can have dramatic impact on vulnerabil-
ity. Additionally, nonchemical stressors can modify the response to exposure or
modify the level of exposure. Many would call inclusion of all these factors impor-
tant to cumulative risk assessment. Environmental exposures are rarely to single
chemical stressors and often this convergence occurs during critical windows of de-
velopment. The need to expand single substance risk assessments to complex envi-
rmental mixtures remains a challenge. This challenge can be addressed with im-
proved problem formulation of the health risk of concern. The reality in assessing
children’s health risk is that there is often a paucity of data for children’s exposures
and hazards. In addition, the lack of data in developing animals for non food use
pesticides limits extrapolation and risk estimation in humans. This lack of data cre-
ates a dependence on default safety and/or uncertainty factors. The contemporary
challenge is how can critical data gaps be filled efficiently and effectively with new
technologies that reduce uncertainty and reliance on cumbersome and expensive
animal testing. (This abstract does not reflect EPA policy).

657 WHERE DO I GO NOW? RATIONAL CAREER
DEVELOPMENT PLANNING FOR EARLY-CAREER
SCIENTISTS.

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Rochester, NY and 2National Center for Environmental Assessment, U.S.
Environmental Protection Agency, Research Triangle Park, NC.

Toxicology training during graduate school and postdoctoral fellowships provides
early-career scientists with a wide array of transferable skills that can be used in
many job sectors, but navigating the all of the possible career options can be a
daunting task. Additionally, finding and preparing for a career path that is right for
yourself is not always easy, particularly when it differs from that of your mentor or
is non-traditional. The majority of students and postdocs are trained in academic
institutions with resources that prepare them for a career in academia. However, a
recent National Postdoctoral Association survey indicated that even though 45% of
the postdocs plan on being a tenure-track faculty member, less than 20% will ob-
tain this position. Therefore, it is important for early-career scientists to gather
ample information and diverse experiences to better prepare them for multiple ca-
reer paths. The first step in this process is to identify transferable skills and translate
them into realistic paths toward a rewarding job. With broad coverage of non-trad-
tional career paths in toxicology, this session will provide early-career scientists
with insight on how to map a career path that fits their passion and skills. Using
an interactive format, speakers will identify tools to utilize in pursuit of navigating
different paths. Discussions will include identifying marketable skills, rational career
planning, networking, and improving marketability. Grant preparation will also be
discussed during a presentation on writing a successful career transition grant appli-
cation. Specifically, the K99/R00 grant program, which has no citizenship restric-
tions, provides support to an individual postdoctoral fellow transitioning to an in-
dependent faculty position.

658 HOW TO IDENTIFY YOUR SKILLS AND PASSIONS.

K. Keefe. Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.
Sponsor: B. Lew

Socrates is quoted as saying, “The unexamined life is not worth living”. It’s proba-
bly safe to say that at some point even Socrates stopped and wondered whether he
had made the right career choice or whether he should be doing something else.
Although used here out of context, Socrates’ words offer instruction to us as scien-
tists in identifying our skills and passions. That instruction is "examine".
Identifying our passions involves examining our values and interests and deciding
which of those will form the basis on which we make decisions about various mat-
ters, including our career, at any particular point in time. This presentation will dis-
cuss the need for continual examination of one’s values and interests, and will pro-
vide reference to resources that individuals can use to help them conduct such
examination. Identifying one’s skills also requires examination; that is, examination
of one’s training and acquired skills, as well as one’s inherent strengths and weak-
nesses. Furthermore, the identification of one’s skills requires one to think broadly
about the capabilities developed to date. This presentation will provide a broad per-
spective on the transferable skills of PhD-level scientists that should be examined,
and will highlight resources available to participants so that they can fully examine
their skill set and improve their ability to market those skills to employers in a wide
range of career settings. The insight derived from such examination should allow
one to make career choices consistent with one’s skills and passions; in turn leading
to a life that one feels is worth living.

659 CAREER PLANNING AND DEVELOPMENT FOR EARLY-
CAREER SCIENTISTS.


Early career development can be looked at as being of two major phases. The first
phase is the formal educational process leading to an awarded degree, postdoctoral
training, and potentially formal certification in a scientific discipline. The second
phase is the informal education of becoming successful in the business of science.
During both phases of your early career it is important to prepare yourself educa-
tionally to capitalize on an opportunity when it presents itself. Since you cannot
predict what the opportunity might be, having a broad understanding of the scien-
tific disciplines with which you will be interacting during your early career is essen-
tial. There is plenty of time in your life to become highly specialized, but doing that
too early can be too limiting. To maximize your training and early career develop-
ment it is important to develop good relationships with multiple mentors. These
mentoring relationships do not, and should not, always be formal but include peo-
ple who can provide you with insights and accumulated wisdom from a variety of
perspectives. A critical attribute of all successful individuals is their ability to know
and understand themselves so that they can honestly assess what strengths and
weaknesses they have that could influence their own career development. For some
people having a formal Individual Development Plan that provides specific training
activities and career goals is effective. The most important thing to remember is
that you must take control of your career; you need to seek out mentors as well as
mentor others, ask for training in areas you desire to understand, and say yes to op-
portunities as they arise. [This abstract of a proposed presentation does not repre-
sent the views or policies of the U. S. EPA.]
the beginning of the academic career. The award is unique among NIH training and career development awards in that it is open to both U.S. Citizens and non-citizens who have postdoctoral appointments at U.S. Institutions. Applicants apply for the K99/R00 while still in the postdoctoral appointment and the award allows the candidate to receive both mentored and independent research support from the same award. Although the research program must have a unifying hypothesis, logical sequence of specific aims to test the hypothesis, and be continuous in time, the application is essentially a composite of two different types of programs. One part is a mentored program and the other part is an independent research effort. This session focuses on the K99/R00 grant program, its provisions and review, and presents tips to address the "hybrid" nature of the application.

L. Lind and D. Devoney.

A POLYMORPHISM IN THE AH-RECEPTOR GENE IS RELATED TO HYPERTENSION AND ENDOTHELIUM-DEPENDENT VASODILATATION.

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Objectives: Since we previously have shown that a dioxin-like agonist for the aryl hydrocarbon receptor (AH, Ah-receptor, Dioxin receptor) increases blood pressure, we here examined the relations between single nucleotide polymorphisms (SNPs) in the AHR gene and prevalence of hypertension in a population-based sample. For SNPs related to hypertension, we also evaluated the relation to endothelium-dependent vasodilation (EDV) as a secondary aim. Material: Blood pressure was measured in 1016 men and women, aged 70 in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Hypertension was defined to be prevalent in individuals on antihypertensive treatment or with blood pressure >140/90 mmHg. EDV was measured by acetylcholine infusion in the forearm and venous occlusion plethysmography. Twenty SNPs in the AHR gene were chosen according to data from the HapMap project and they were genotyped using the Illumina Golden Gate assay. Results: Of the 20 SNPs analysed, six showed an association with hypertension with a p-value < 0.05. The strongest association signal was found for the SNPs rs17137566 (p=0.0038 following gender-adjustment), where a dose-response for the number of A alleles and the prevalence of hypertension was seen, with the rare allele being protective against hypertension. This genotype was also related to EDV in the expected way (p= 0.018 following gender-adjustment). Conclusion: The present study showed that genetic variation in the AHR gene is related to hypertension, as well as to endothelium-dependent vasodilatation in resistance vessels, suggesting that the AHR could be involved in blood pressure and resistance vessel regulation.


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There are many studies on non specific building related symptoms and indoor environment. Most of them are cross-sectional and several deals with occupational environments. Most of them are cross-sectional and several deals with occupational environments. There are many studies on non specific building related symptoms and indoor environment. Most of them are cross-sectional and several deals with occupational environments. Most of them are cross-sectional and several deals with occupational environments. There are many studies on non specific building related symptoms and indoor environment. Most of them are cross-sectional and several deals with occupational environments.

Background: A relationship between blood pressure (BP) and high level blood lead concentrations has been reported and this relationship appeared to be influenced by the sex and/or race of the participants. The objective of this study was to evaluate whether low blood-lead levels (BLLs ≤ 10 μg/dL) were associated with BP among adults aged 20 years and older in the U.S. population. Methods: We analyzed data from NHANES 1999–2006 participants aged 20 years or older who were white non-Hispanic or black non-Hispanic with BLLs ≤ 10 μg/dL. Outcome variables were systolic and diastolic BP measurements and hypertension status. Results: In multiple regression analyses, BLLs were significantly correlated with higher systolic BP among black men and women, but not white, participants. The change in systolic BP associated with a two-fold increase of blood lead concentration was 1.59 mmHg in black men, and 1.66 mmHg in black women. BLLs were significantly associated with higher diastolic BP among white men and women and black men, but not among black women. Black men with BLLs between 5.01 and 10 μg/dL had an adjusted prevalence odds ratio of 1.98 (95% CI: 1.11–3.50) to have hypertension compared with black men with BLL < 5 μg/dL. Conclusions: Low-level BLLs among black non-Hispanic men participants in the NHANES 1999–2006 were associated with increased systolic and diastolic BP and higher hypertension risk. Despite the decline in blood-lead concentrations in the U.S. population, lead exposure remains a major public health priority and is still a health risk.

PREVALENCE OF NEUROPATHIC CAMPYLOBACTER JEUNI (CJ) ON COMMERCIAL BROILER CHICKEN PRODUCTS.

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Campylobacteriosis is the most common antecedent infection leading to Guillain Barre Syndrome (GBS), the most common cause of acute paralysis in the US. Exposures to CJ are most due to poultry consumption; we have reported that poultry workers are also at risk of CJ exposure and are 30 times more likely to report symptoms consistent with inflammatory peripheral neuropathy. Overall, while persons with campylobacteriosis have 100 times the risk of GBS and for campylobacteriosis to report symptoms consistent with inflammatory peripheral neuropathy. Overall, while persons with campylobacteriosis have 100 times the risk of GBS


Epidemiologic studies indicate an association between formaldehyde exposure and lymphohematopoietic malignancies (LHP) in a range of occupational cohorts as well as in case-control studies. Early studies reported the strongest associations for all leukemia and for myeloid leukemia as a specific subtype. There has been considerable debate on the biological plausibility of formaldehyde-induced leukemia, specifically for myeloid leukemia. Therefore, available data were analyzed by etiologically-related subtypes in an effort to better understand which disease, or disease groupings, are consistently associated with formaldehyde exposure, thereby providing a better basis for judging biological plausibility. Data from two published cohorts were available for subtype analysis; a study of garment workers (Hayes et al., 1990) and the NCI study of industrial workers (Beane-Freeman et al., 2009). Unadjusted odds ratios for two novel disease groupings are presented for each study: 1) all LHP, less myeloid leukemia (ICD 8 codes 200-204, 206-209), and 2) solid tumors of lymphoid origin (ICD 8 codes 200-203). For both cohorts, associations between formaldehyde exposure and LHP malignancies (ORs 1.39(1.05-1.84) and 1.36(1.04-1.78) were retained without myeloid leukemia (ORs of 1.35(0.99-1.85) and 1.30(0.97-1.74) in garment workers and the peak exposure group in the NCI industrial cohort respectively. Similar associations were also found when considering only solid tumors (OR 1.24(0.84-1.84) and 1.32(0.92-1.89)). These data suggest that observed associations of increased LHP are not due solely to increases in myeloid leukemia as was previously believed. The finding of positive association for the lymphoid subtypes, especially Hodgkin's Disease, multiple myeloma and other diseases which arise from mature lymphocytes, reframes the debate on the biological plausibility of formaldehyde-induced LHP malignancies.

Disclaimer: This abstract does not necessarily reflect EPA policy.
the structure of surface lipooligosaccharides (LOS) of CJ results in epitopes sufficiently similar to membrane glycolipids on mammalian peripheral neurons to stimulate autoimmunity by molecular mimicry. These neuropathic LOS structures are associated with polymorphisms in bacterial sialytransferases. In this study, we sought to determine the prevalence of neuropathic CJ strains on commercial poultry products to assess risks of exposure to food consumers as well as poultry workers, and to determine if other factors modulate odds of GBS after poultry-borne infection. CJ was isolated from poultry products purchased at retail outlets in Baltimore in 2005-7. Neuropathic strains, defined by LOS and sialytransferase polymorphisms, were identified by PCR. We found close agreement between the prevalence of neuropathic strains in poultry isolates and those reported in persons with C. jejuni enteritis, confirming the predominant role of poultry in human CJ infection. The prevalence of neuropathic strains in poultry isolates was relatively high and significantly greater than 10%, which indicates that additional factors (probably host related) also modulate risks of GBS following CJ infection. The high prevalence of neuropathic CJ in poultry products reinforces concerns for occupational exposures among poultry farmers, farm workers, and processing plant workers.


A. Salem Sreensivasan1, S. A. Khan2, S. Gwalney-Brant2, M. R. Slater1 and V. R. Beasley1. 1Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; and 2ASPCA APCC, Urbana, IL.

Information on exposure and management of Mixed Amphetamine Salts (MAS) toxicosis in companion animals is limited. This study characterizes adverse clinical effects in cats exposed accidentally to MAS and discusses its treatment and management. Historical, epidemiologic, and clinical information involving MAS incidents in cats were retrieved from the computerized medical record database of the ASPCA Animal Poison Control Center. Only witnessed or observed incidents involving single animal with high and medium assessments of toxicosis and those that did not develop clinical signs were included in the study. Age, gender, breed, season of year, and outcome frequencies were determined. One hundred and fifty two incidents met the case criteria. Summer months showed least number of incidents. Male cats (56.6%) were more frequently involved compared to female cats (42.8%). Incidents in adult cats were higher (65.8%) compared to juvenile (31.6%) and elderly (2.6%) cats. Most incidents involved mixed breed (90.8%) cats. Out of 12.5% cases (n= 19) in which outcome data was available, 10% cats made full recovery, 2.0% did not develop any clinical signs, and 0.7% died. Most clinical cats showed CNS, cardiovascular (CV), and behavioral effects following ingestion. Disorientation, lethargy, hyperthermia, tremors, circling, tachycardia, hypertension and hypotension were the most common CNS and CV effects. Management and treatment included decontamination, supportive care with IV fluids, thermoregulation, and treatment with specific serotonin antagonist cyproheptadine. The minimum dose at which clinical signs developed was 0.5 mg/kg and one death was reported at 2.5 mg/kg. This data shows that cats can show serious CNS, CV, and behavioral clinical signs following accidental MAS ingestions; however, good recovery is possible with prompt and aggressive veterinary care.

670 META-ANALYSIS OF AIRWAY HYPER-RESPONSIVENESS IN ASTHMATICS AFTER NITROGEN DIOXIDE EXPOSURE.


Many studies over the past three decades have investigated the effects of nitrogen dioxide (NO2) on airway hyper-responsiveness (HHR) in asthmatics, yet no conclusive picture of the possible relationship between the two has emerged. NO2 is an environmental pollutant that is considered to be of potential harm to environmental and human health at certain exposure levels; as such, the U.S. EPA establishes air quality standards for NO2, as part of the Clean Air Act. Of late, interest in NO2 has increased as the U.S. EPA considers revising the National Ambient Air Quality Standards (NAAQS) for NO2. We conducted a systematic literature review along with meta-analyses and meta-regressions of controlled exposure studies of the effects of 0.1 to 0.6 ppm NO2 on AHR in response to airway challenges in asthmatics. Our analytical approach used several effect measures for AHR: a change (in NO2 vs. air) in (1) the provocative dose of a challenge agent necessary to cause a specified change in lung function (PD), (2) the change in FEV1, after an airway challenge, and (3) the fraction of subjects with increased AHR. The meta-effect estimates were, for change in provocative dose using group data: -27.0% (95% CI: -35.6%, -18.5%); change in provocative dose using individual data: -11.5% (95% CI: -21.6%, -1.4%); change in AFEV1: -1.75% (95% CI: -3.29%, -0.21%); and fraction of subjects affected: 0.58 (95% CI: 0.5, 0.63). Although the effect estimates from meta-analyses of the complete datasets are statistically significant, they are small relative to clinically relevant effects. There are no exposure-response associations for any effect estimates based on linear meta-regressions. In addition, our analyses indicate that, to the extent the effects observed are associated with NO2 exposure, they are sufficiently small such that they do not provide evidence that NO2 has a significant adverse effect on AHR at concentrations up to 0.6 ppm.

671 PHARMACOLOGICAL INHIBITION OF TGFβ1 SIGNALING ENHANCES MALIGNANT PROGRESSION OF CHEMICALLY INDUCED SKIN CANCERS THROUGH CHANGES IN INFLAMMATORY RESPONSE.

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Transforming growth factor β1 (TGFβ1) is important in the regulation of development, inflammation and carcinogenesis. Small molecule inhibitors of the TGFβ1 type 1 receptor kinase (ALK5) like SB431542 (SB) have been developed to inhibit the TGFβ1 signaling pathway, showing potential as anti-inflammatory and anti-cancer agents. However, the duality of TGFβ1 signaling in cancer as both a tumor suppressor and oncogene suggests that long term pharmacological intervention in this pathway could provoke rather than inhibit tumor formation. To test the role of TGFβ1 in the development of skin tumors we treated FVB/n mice with SB in a 2-stage chemical carcinogenesis assay. ALK5 inhibition decreased papilloma forma-
trophil infiltration, a significant increase in hyperplasia, and no differences in proliferation or apoptosis. In agreement, there was a 5-fold decrease in KC expression and inhibition of MIP2, while there was no change in KC and TNF. In FVB/n keratinocytes we found a 2.5-fold decrease in MIP2 expression and inhibition of MIP2, while there was no change in MIP2, s100a8/9, and TNF. In agreement, there was a 5-fold decrease in KC expression, with no change in MIP2, s100a8/9, and TNF. Increased AKT activation. Inhibition of AKT activation abolishes CsA-promoted growth and survival, indicating that AKT hyperactivation is essential for the enhanced growth and survival of CsA-treated cells. In addition, mTOR signaling as a known AKT downstream target is required for CsA-enhanced growth and survival. Taken together, we have identified PTEN/AKT pathway as new molecular targets of CsA in epidermal keratinocytes, suggesting a previously unknown mechanism in CsA-enhanced skin carcinogenesis. Our findings challenge assumptions about how CsA-associated tumors arise in skin.

GEOGENE EXPRESSION PROFILE OF THE BONE MARROW AFTER BENZENE EXPOSURE IN C57BL/6 AND C3H/HE MICE: ELUCIDATION OF STRAIN DIFFERENCE BY MICROARRAY STUDY WITH RECIPROCAL GENE EXPRESSION PROFILE, THE COMMON GENE EXPRESSION AND THE STOCHASTIC GENE EXPRESSION.

673 IMMUNOSUPPRESSIVE CYCLOSPORIN A ACTIVATES AKT IN KERATINOCYTES THROUGH PTEN SUPPRESSION: IMPLICATIONS IN SKIN CARCINOGENESIS.

Cyclosporin A (CsA) is the most commonly used immunosuppressant in organ transplantation. It is an effective regulator of AKT activation, is reduced significantly upon CsA treatment in TCL5 alters tumour progression, suppressing development of benign lesions, yet enhancing malignant conversion through inhibition of neutrophil chemokines.

C/EFP-T and C/EFP-B are kZIP transcription factors that are determinants of skin tumorigenesis. Epidermal specific C/EFP-T knockout mice are highly susceptible to carcinogen-induced skin tumorigenesis while epidermal specific C/EFP-B are resistant. While there is a significant tumor phenotype in these mice, deletion of either isoform alone has only a minor effect on epidermal hyperplasia. Thus, the function of C/EFP-T and C/EFP-B in skin is unknown. An understanding of the function of C/EFP-T and C/EFP-B within the skin may ultimately provide insight into their role in skin tumorigenesis. To address possible functional redundancy and reveal functional roles of C/EFP-T and C/EFP-B within the skin, mouse models were developed in which either family member could be acutely ablated alone or together in the epidermis, hair follicles and sebaceous glands of adult mice. Deletion of either family member in the epidermis or sebaceous glands had little effect on skin tumorigenesis. Epidermal specific C/EFP-α is a resist-
We propose that in adult epidermis, C/EBPα and C/EBPβ have essential roles in the basal to spinous cell transition and influence cell cycle withdrawal of basal keratinocytes and are required for sebocyte differentiation. C/EBPα and C/EBPβ have cooperative overlapping roles in keratinocyte and sebocyte differentiation but independent effects on skin tumorigenesis.

**PL 676 TUMOR-ELICITED ALVEOLAR MACROPHAGES PRODUCE IGF-1 TO AUGMENT NEOPLASTIC EPITHELIAL PROLIFERATION IN AN ERK1/2 DEPENDENT MANNER.**

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**Rationale:** After lung cancer is initiated by environment toxins such as those found in cigarette smoke, the resultant chronic inflammation promotes growth of these initiated cells. In particular, alveolar macrophages increase dramatically during tumor progression. In a mouse model of human lung cancer, tumor growth is inhibited if macrophages are depleted, and exacerbated by chemically-induced inflammation. We have determined molecular mediators of this macrophage-assisted tumor growth. Methods: Bronchoalveolar lavaged (BAL) macrophages from naïve or tumor-bearing animals were cultured to generate macrophage conditioned media (M0CM). To directly assess effects of primary lung macrophages on epithelial cell proliferation, macrophages were co-cultured with primary tumor cells isolated or stable non-tumorigenic and neoplastic lung epithelial cell lines in vitro. Results: Neoplastic lung cell proliferation was enhanced in an Erk1/2 and Akt dependent manner when co-cultured with macrophages from either naïve or lung tumor-bearing mice. The TH2 cytokine IL-4 can polarize macrophages to an M2 pro-growth state. M0CM from IL-4 treated BAL macrophages stimulated epithelial proliferation more than media taken from untreated macrophages. Soluble protein growth factors present in M0CM were screened by ELISA. Insulin-like growth factor 1 (IGF-1) production was stimulated by IL-4 treatment. IGF-1 concentration in BAL fluid was 3x greater in tumor-bearing mice than naïve. Pharmacological inhibition of IGF receptor (IGFR) decreased basal neoplastic cell growth, and prevented growth stimulation by macrophage co-culture, M0CM, or direct IGF-1 addition. Conclusions: Lung tumors conscript and re-educate alveolar macrophages to increase IGF-1 production, which acts through IGFR and Erk1/2 to enhance neoplastic proliferation. (Supported by CA33497 and CA15532; Friz, J.M. is a American Foundation for Pharmaceutical Education Fellow)

**PL 677 CR(VI) EXPOSURE INDUCES TELOMERE LOSS AND DEFECTS.**

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Telomeres cap and protect the chromosome ends from being recognized as DNA breaks, and consist of guanine-rich DNA repeats coated by specialized proteins. Critically short or deleted telomeres cause cellular genomic instability, senescence or death that is associated with disease, aging and cancer. Telomere dysfunction contributes to Werner syndrome (WS) pathology, a premature aging disorder marked by increased cancer. Previous reports indicate the protein defective in WS (WRN) has roles in proper replication and repair of telomeres. We previously showed that WRN functions in repair of replication-associated DNA damage induced by the environmental carcinogen hexavalent chromium (Cr(VI)). Therefore, we hypothesized that Cr(VI) exposure may induce telomere damage that interferes with telomere replication and leads to telomere loss and defects. We found WS cells are hypersensitive to Cr(VI), but telomerase expression significantly reduces Cr(VI) sensitivity. This suggests that telomerase, which extends truncated telomeres, can compensate for WRN roles in protection against Cr(VI) induced telomere damage. To measure telomere damage directly we examined the localization of gH2AX, a marker for DSBs, at telomeres using a telomere probe. Cr(VI) exposure induced a dose and time dependent increase in the co-localization of gH2AX at telomeres, that was significantly increased compared to ionizing radiation, which generates random chromosome breaks. Furthermore, cellular exposure to Cr(VI) caused increased telomere loss and defects as observed in chromosome spreads. We are currently testing whether WRN protein functions in the prevention or repair of Cr(VI) induced telomeric damage. We observed that WRN protein localizes to telomeres after Cr(VI) exposure. Together, this study provides novel evidence that an environmental pollutant can induce telomere damage, which may contribute to environmentally relevant diseases including cancer. Supported by NIEHS grant ES015051-01 (FLO).

**PL 678 PERSISTENT NUCLEAR-CYTOPLASMIC ERK OSCILLATIONS ARE DEREGULATED BY TOXIC INSULT.**


The regulation of protein kinase activity by toxicological agents plays an important role in pathophysiological processes. Many toxicants exhibit different toxicological profiles at low and high concentrations and protein kinase regulation often follow these trends - showing increased or decreased kinase activity as a function of toxicant concentration. We have compared the regulation of cell transformation responses and complex patterns of kinase behavior (oscillations) in JB6 cells treated with basic fibroblast growth factor (bFGF) and tumor promoting phorbol ester (TPA). Interestingly, while both bFGF and TPA induce anchorage-independent growth (AIG) of JB6 cells, bFGF-dependent AIG is reversible while TPA-dependent AIG is irreversible. This pattern directly correlated with the appearance of persistent nuclear-cytoplasmic ERK oscillations in response to bFGF stimulation, while TPA induced persistent ERK activation without observable oscillations. We have demonstrated that low dose ionizing radiation (10 cGy, X-ray) induces irreversible AIG in the JB6 model. X-irradiation did not induce ERK oscillations and induced bFGF-dependent ERK oscillations in the absence of hypoxia. The resulting complex regulation of the ERK pathway that parallels acquisition of irreversible AIG properties as stimulated by established carcinogens. Deregression of ERK oscillations by low dose radiation indicates that regulated kinase oscillations are highly sensitive to toxic insult.

**PL 679 THE ROLE OF HYPOXIA IN 2-BUTOXYETHANOL-INDUCED HEMANGIOSARCOMA.**

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To understand the molecular mechanisms underlying compound-induced hemangiosarcasmas in mice, and therefore their human relevance, a systematic biological approach was undertaken using transcriptomics and Causal Network™ Modeling from tumors treated with 2-butoxyethanol (2-BE), a hemangiogenic agent that induces hemangiosarcomas in mice. We hypothesized that the hemolysis induced by 2-BE would result in local tissue hypoxia, a well-documented trigger for endothelial cell proliferation leading to hemangiosarcoma. Gene expression data from bone marrow, liver, and spleen of mice exposed to a single dose (4b) or 7 daily doses of 2-BE were used to develop a molecular model of hemangiosarcoma. The resulting mechanistic model confirms previous work proposing that 2-BE induces macrophage activation and inflammation in the liver. In addition, the model supports local tissue hypoxia in the liver and spleen, coupled with increased Epo signaling and erythropoiesis in the spleen and bone marrow, and suppression of mechanisms that contribute to genomic stability, events that could be contributing factors to hemangiosarcoma formation. Finally, an immunohistochemistry method (Hypoxyprobe™) demonstrated that tissue hypoxia was present in the spleen and bone marrow. Together, the results of this study identify molecular mechanisms that initiate hemangiosarcoma, a key step in understanding safety concerns that can impact drug decision processes, and identified hypoxia as a possible contributing factor for 2-BE-induced hemangiosarcoma in mice.

**PL 680 THE FUNGAL PRODUCT AFLATOXIN B1 AFFECTS VISUAL DEVELOPMENT IN LARVAL ZEBRAFISH.**

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Aflatoxin B1 (AFB1) is produced by the fungal genus Aspergillus and has been found as a contaminant in maize and peanut crops, particularly in tropical regions with poor harvesting techniques. AFB1 has been shown to cause a wide range of biological effects including toxicity, carcinogenicity, genotoxicity and impairment of the reproductive system. AFB1 has also been localized in the retina of embryonic mice after injection. This study examined the effects of sub-lethal AFB1 exposure on the morphology and visual behaviors of developing larval zebrafish (Danio rerio). Larvae were exposed to either a vehicle or to doses of AFB1 ranging from 1.0 to 12.5 µg/ml holding water for 48 hours beginning one day post-fertilization (dpf). Mortality, gross morphology (total length and eye diameter) and visual behavior were examined at 5 dpf. Visual behavior was evaluated by examining photo-
tactic response and optokinetic response to a striped, rotating drum. Mortality increased significantly with increasing aflatoxin dosage. This study is the first to link eye diameter did not change when differences in total length were accounted for. The number of fish showing the appropriate positive phototaxis decreased significantly with increasing aflatoxin dosage. Similarly, the percentage of fish successfully tracking the rotating cylinder with their eyes in the optokinetic challenge decreased significantly with increasing aflatoxin dosage. This study is the first to link AFB1 toxicity with changes in the visual behavior of a vertebrate. The behavioral findings suggest damage to the eye or visual processing centers and may provide insight into the pathology of AFB1 toxicity on visual system development in vertebrates. As a model organism, zebrafish might be used to screen for AFB1 effects or provide insight into the pathology of AFB1 toxicity on visual system development in vertebrates. As a model organism, zebrafish might be used to screen for AFB1 effects or provide insight into the pathology of AFB1 toxicity on visual system development in vertebrates.

FETAX to be a useful screening assay to evaluate the teratogenic potential of drug candidates before entry into development.

For 10 years, the FETAX (Frog Embryo Teratogenicity Assay Xenopus) has been used at sarofin-aventis as a routine screening test for candidate compounds. To date, out of 400 candidates tested in this assay, around 50 were also evaluated in vivo embryotoxicity studies in rats and rabbits according to standard ICH protocols. Recently, 26 out of these compounds were also tested using the DarT. We will present and compare data obtained with these compounds and discuss correlations between in vitro screening and in vivo study results. For the FETAX (conducted in house), Xenopus laevis embryos obtained by in vitro fertilization, were exposed to 5 to 96 hours post fertilization (hpf) to various concentrations of the test material dissolved in 0.01% DMSO. For DarT (conducted at 2 different CROs), Zebrafish embryos obtained by natural mating, were exposed from 6 to 120 (or 4 to 96) hpf in 6-or 24 well plates with drug in DMSO (0.3% max). Developmental effects were evaluated at the end of the culture period using similar endpoints for both assays (i.e. heart, circulation, brain, jaw, tail malformations and body length). A teratogenic index (TI) representing the ratio between the 50% lethal concentration and the concentration causing 50% of malformed larvae, was established for each drug. In these screening assays, a compound was considered potentially teratogenic when its TI was greater than 1.2 (FETAX) or 1 (DarT). Using our complete data set, the predictivity of the FETAX was close to 70% (positive and negative compounds correlating with in vivo results). When the compounds, concluded inconclusive based on the TI calculation but showing the presence of rare malformations, are counted positive this predictivity reaches 80%. When looking at the 26 compounds tested in both screening assays, the predictivity was 50% for DarT and 72% for FETAX. In conclusion, we consider the FETAX to be a useful screening assay to evaluate the teratogenic potential of drug candidates before entry into development.

In zebrafish, TCDD impairs cartilage morphogenesis but does not chondrocyte differentiation. TCDD-induced jaw malformation is AHR2-dependent and secondary to reduction in sox9b transcript abundance in the larval jaw. Sox9b encodes a transcription factor critical for chondrogenesis. The mechanism by which TCDD downregulates transcription of sox9b is not known. We hypothesize that TCDD inhibits transcription of sox9b by decreasing its positive regulator zebrafish. We cloned a sox9b promoter fragment that is 2570 bp upstream of the sox9b transcriptional start site (-2570/0). We made several 5’ deletions to the -2570/0 sox9b clone to make -2067/0, -1060/0 and -712/0 clones. These clones were inserted in front of the egfp gene to produce transgenic zebrafish lines. We have not identified a F0 carrier for the -2067/0 or -1060/0 sox9b reporters. The F1 generation of both -2570/0 and -712/0 embryos express EGFP in the jaw, heart, pectoral fin, eye, and brain. However, -2570/0 transgenics express EGFP at greater levels in the jaw, pectoral fin and notochord than the -712/0 reporter fish. This restriction of reporter activity to different tissues suggests that the -2570/0 sox9b reporter construct contains regulatory DNA sequences that are functional in the jaw, pectoral fin and notochord. To test the effect of TCDD on reporter activity, we exposed -2570/0 and -712/0 transgenic embryos to TCDD and vehicle at 24 hpf and measured egfp transcript abundance at 96 hpf. TCDD downregulated egfp mRNA in -2570/0 embryos but not in -712/0 embryos. Importantly, the fold reduction in egfp mRNA was comparable to that produced by TCDD on sox9b mRNA. However, tgf -712/0/tcn sox9b(gefp)/+ embryos exposed to TCDD showed no change in egfp mRNA at 96 hpf. These preliminary results suggest that the promoter sequence between -2570/0 and -712/0 is needed for inhibitory effects of TCDD on sox9b transcription. The exact location within the promoter or the mechanism by which TCDD inhibits sox9b transcription remains to be identified.

684 UNRAVELING THE ROLE OF THE AHR IN DIFFERENTIAL DEVELOPMENTAL TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN ZEBRAFISH.

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously present in the environment as products of incomplete fossil fuel combustion, and are of increasing concern to human health, particularly in urban areas. Much research has focused on PAHs that cause toxicity via activation of the aryl hydrocarbon receptor (AHR), which regulates expression of a number of responsive genes such as CYP1A. PAHs induce developmental toxicity in animal models, and while some developmental effects are AHR dependent, others such as cardiac toxicity can be induced by PAHs via AHR-independent mechanisms. Understanding the mechanisms by which structurally-similar groups of PAHs induce toxicity is essential in assessing risk to human health. Zebrafish were exposed to the model PAHs pyrene, benz(a) anthracene and dibenzothiophene over the course of development, and gross morphological assessments were conducted to establish appropriate sensitivity windows for comparative whole-genome gene expression studies. These 3-4 ring PAHs induce distinct biological responses in zebrafish that are AHR2 and CYP1A dependent, AHR2 dependent, and AHR2 independent, respectively. In order to better investigate the role of the AHR in PAH-induced developmental toxicity, a mutant zebrafish, abr2hu3335 was created using TILLING (Targeting Induced Local Lesions IN Genomes). The abr2hu3335 line has a point mutation in AHR2 residue 534, in the C terminal domain, resulting in a premature stop codon. We generated a homologous knockin of abr2hu3335 mutants, and confirmed functional knockdown of AHR2 by immunohistochemistry of CYP1A expression. Exposure from 48-120hpf (hours post fertilization) with known AHR2 ligands failed to induce CYP1A expression in mutant larvae, while wild-type zebrafish exhibited robust expression. The mutant AHR2 zebrafish line promises to be a valuable tool for unraveling the role of AHR2 in the developmental toxicity of PAHs and other putative AHR ligands. This research was supported by NIEHS grants P30ES050210, P42ES016465 and T32ES07060.
PL 685 COMBUSTION-DERIVED POLYNUCLEAR AROMATIC HYDROCARBON (PAHs) INTERFERE WITH ZEBRAFISH EMBRYO DEVELOPMENT AND ARE RETAINED AFTER EXPOSURES END.

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Rationale: Incomplete combustion of hydrocarbons during a) routine flaring at refineries and other petrochemical facilities, b) transportation activities, and c) operation of heavy machinery produces much of the environmental burden of polynuclear aromatic hydrocarbons (PAHs). Commercial rubber products are produced from 1,3-butadiene (BD; CH2=CHCH=CH2). Incomplete combustion of BD produces black smoke—composed of butadiene soot (BDs) nanoparticles—similar to smoke from flares at refineries. PAHs adsorbed to the surfaces of BDs nanoparticles cause oxidative stress and inflammation in lungs of mice following BDs inhalation. Individual PAHs can interfere with zebrafish (Zf) development. Our goal here was to investigate the effects of mild BDs exposure on development of Zf embryos. Methods: From 24–72 hr post-fertilization we exposed Zf (D. rerio; AB strain) embryos to BDs (6–600 µg/ml) sprinkled onto the surface of Zf medium. Results: We observed PAH-associated fluorescence in the yolk sac of embryos and in the gastrointestinal tract, endothelial cells of blood vessels, and eyes of larvae. PAH-associated fluorescence persisted in Zf at least 11 days post-exposure. Dose-related developmental changes included hatch time delays, pericardial edema, lordosis, abdominal swelling and decreased heart rate. PAH metabolism and associated gene expression changes are currently being assessed. Conclusion: Brief, low-level exposures of Zf embryos to petrochemical combustion particles induce developmental changes and results in extended retention of PAHs. (Allen Bui was a Summer Scholar supported in part from a Grant to the LSU College of Basic Sciences from the Howard Hughes Medical Institute Biomedical Education Program).

PL 686 PLEIOTROPIC NATURE OF ETHANOL ACTION IN MEDIKA EMBRYOGENESIS.

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Fetal alcohol spectrum disorder (FASD) is a group of birth defects characterized by abnormal facial features, growth retardation, and central nervous system malformation and dysfunction. These defects are irreversible if a pregnant woman consumes alcohol during a critical period of fetal development. While the phenotypic expressions of FASD are well known, the molecular mechanisms of this developmental disorder are yet unknown. Moreover, the dose and the period of embryogenesis most sensitive to ethanol are also unknown. We used Japanese medaka (Oryzias latipes) embryo to determine a critical dose and a developmental stage sensitive to ethanol, and to evaluate oxidative stress as a possible cause of FASD. Previously, we determined that medaka embryos exposed to ethanol at different developmental stages for 48 h have microcephaly with reduced ethmoid plate (EP), disrupted trabecular (TC) and polar cartilages (PC), and loss of mediolateral polarity of the neurocranium (NRC). In the present experiments medaka embryos of 0-6 dpf development were exposed to 300 mM ethanol either for 24 h and allowed to hatch, or exposed for 48 h, then used for the determination of reactive oxygen species (ROS) as an index of oxidative stress. The hatchlings were stained in Alcan Blue and the linear length of NRC, EP, TC and PC were measured. It was observed that ethanol is able to reduce the linear lengths of NRC, EP and TC in embryos of 24–96 hpf, however, PC remained unaffected. The ROS was undetectable in embryos in early stages (0-2 dpf) of development, but detectable in the embryos once the heart started beating. A gradual increase of ROS was observed with the advancement of morphogenetic the highest level was achieved in hatchlings. Embryos (0-4 dpf) treated with ethanol (300 mM) for 48 h showed no alteration in ROS level. Moreover, embryos preincubation are more resistant to BD than ones with circulation. Biochemical analyses are unable to establish an effect of BD in total protein, RNA and DNA contents and in the expression of emx2, en2, io3, otx2, shh, wnt1 and zic-5 mRNAs in circulating embryos. However, in preincubating embryos, total RNA content of shh mRNA expression were reduced after BD treatment. By using subtractive hybridization, we are able to demonstrate that gata2 mRNA was differentially expressed in the circulating embryos after BD treatment. Further analysis identified the rapid expression of gata2 mRNA followed by preproenodishel1 (edn1) mRNA by BC which might be able to induce vasocstriction and dysfunction in medaka embryogenesis by BC is probably mediated through Gata2-Edn1 signaling pathway.

PL 687 TERATOGENIC EFFECTS OF BLUE COHOSH (CAULOPHYLLUM THALICTROIDES) IN JAPANESE MEDIKA (ORYZIAS LATIPES) ARE MEDIATED THROUGH GATA2/EDN1 SIGNALING PATHWAY.

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Blue cohosh (Caulophyllum thalictroides) (BC) has been used widely to induce labor and to treat other uterine complications. However, the safety and effectiveness of this herbal product have not yet been evaluated by the Food and Drug Administration (FDA). Several reports indicated that the root extract of BC is a teratogen, and by some unknown mechanisms, is able to induce cardiovascular malfunctions in newborn babies. To understand the mechanism we used Japanese medaka (Oryzias latipes) embryo-level development as the experimental model and methanolic extract of root of BC as the teratogen. The embryo mortality, hatching efficiency, morphological abnormalities in craniofacial and cardiovascular systems and several biochemical parameters are considered for the evaluation of BC toxicity. Our results indicate that BC is able to disrupt cardiovascular and craniofacial cartilage development in medaka embryos in a dose and developmental stage-specific manner. Moreover, embryos preincubation are more resistant to BD than ones with circulation. Biochemical analyses are unable to establish an effect of BD in total protein, RNA and DNA contents and in the expression of emx2, en2, io3, otx2, shh, wnt1 and zic-5 mRNAs in circulating embryos. However, in preincubating embryos, total RNA content of shh mRNA expression were reduced after BD treatment. By using subtractive hybridization, we are able to demonstrate that gata2 mRNA was differentially expressed in the circulating embryos after BC treatment. Further analysis identified the rapid expression of gata2 mRNA followed by preproenodishel1 (edn1) mRNA by BC which might be able to induce vasocstriction and dysfunction in medaka embryogenesis by BC is probably mediated through Gata2-Edn1 signaling pathway.

PS 688 GAMMA-TOCOPHEROL QUINONE IS NOT MUTAGENIC IN A VARIETY OF IN VITRO AND IN VIVO GENOTOXICITY ASSAYS.

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alpha-Tocopherol quinone (α-TQ), a coenzyme Q10 analog demonstrated to improve mitochondrial function in vitro, is in clinical trials for treatment of various mitochondrial diseases. As with other pharmaceuticals, the drug product contains trace impurities. In this case, the fully substituted α-TQ may contain gamma-tocopherol quinone (γ-TQ), a partially substituted quinone considered to be structural alerting for mutagenicity due to its potential to form Michael adducts with cellular nucleophiles. Also, γ-TQ has been reported to be an arylating agent and highly mutagenic in AS52 cells. Therefore, as part of the qualification of the impurity, neat γ-TQ and γ-TQ spiked into α-TQ at levels in excess of those present in drug product were evaluated in an expanded genotoxicity battery performed in compliance with ICH and OECD guidelines and GLP regulations. α-TQ spiked and γ-TQ were uniformly negative in three in vitro assays: a 5-strain bacterial reverse mutation (Ames) assay up to 5000 µg/plate ±s; a chromosome aberration assay in purified human peripheral blood lymphocytes, treated for 3 hours ±s and 24 hours ~s, up to cytotoxic toxic limits; and in the AS52/XPRT assay, up to cytotoxic or solubility limits ±s, performed under significantly more rigorous conditions than previously reported. α-TQ spiked and γ-TQ also were negative in an in vivo mouse micronucleus assay up to the MTD (administered intravenously twice ~24 hours apart). Thus, γ-TQ has been demonstrated to be negative in the two-test in vitro genotoxicity battery generally recommended for qualification of potentially mutagenic drug impurities, in an in vivo micronucleus assay as well as in the lone test system previously purported to have produced a positive response. It is therefore concluded that γ-TQ poses little or no mutagenic risk.

PS 689 PIG-A GENE MUTATION IN MAMMALIAN RED BLOOD CELLS.


A number of protein markers are linked to the outer surface of mammalian cells by glycosyl phosphatidylinositol (GPI) anchors. Inactivating mutations in the endogenous Pig-A gene, the only direct linked gene in the GPI biosynthesis pathway, disrupt GPI synthesis and cause a deficiency in multiple GPI-anchored proteins (GPI-APs). Pig-A mutant cells can be identified phenotypically and enumerated rapidly using high throughput flow cytometry. Due to the conserved nature of GPI and its synthesis pathway, models for the detection of mutation in the Pig-A gene have been developed for various species and for multiple cell types. We determined the frequency of spontaneous Pig-A mutant red blood cells (RBCs) from the peripheral blood of mice, rats, sheep, monkeys, and humans. Also, we determined RBC Pig-A mutant frequencies (MFs) induced by treatment with compounds of toxicological interest. Mice were treated with N-ethyl-N-nitrosourea (ENU), rats were treated with ENU methylphenidate (MPH) and muren, monkeys were treated with Cys-Pt and cyclophophamide (CP). The spontaneous Pig-A/MP-deficient RBC MF was low in all species (under 10×108/). However, 2% of nearly 100 healthy human volunteers had un-
usually high spontaneous PIG-A MFs. Similar outliers were not found in a large number of rats or a limited number of monkeys or mice. ENU treatment significantly elevated RBC MF in mice, rats, and monkeys. The liver-specific carcinogen, furan, was not mutagenic in rats and MPH was not mutagenic in rats or monkeys. Shortly after administration of CP or CisPt to human cancer patients, there was no evidence of increased PIG-A MF. Results from monitoring the treated patients over a 6-month period will be shown. Flow cytometric detection of GAPI-A-deficient cells is a rapid and inexpensive tool for detection of in vivo somatic cell mutation. The assay can be used as a preclinical genotoxicity safety assessment as well as in the early stages of clinical trials and in human biomonitoring studies, especially if a pre-exposure MF can be established.

An in vivo mutation assay has been developed based on the flow cytometric enumer- ation of GPI anchor-deficient rat erythrocytes. With this method, anti-CD59-PE and SYTO 13 dyes are used to label leukodepleted blood samples, and flow cytometry is used to determine the frequency of CD59-negative erythrocytes and reticulocytes. The experiments described herein were designed to evaluate the transferability of the method. For these studies, each of four performance sites treated male Sprague Dawley rats at 7-8 weeks of age for three consecutive days with N-ethyl-N-nitrosourea (ENU) via oral gavage. All sites studied 0, 20, and 40 mg/kg/day (n = 5 per group). In addition, two laboratories studied ENU at 4 mg/kg/day. Serial blood samples were collected over time, at a minimum on Days -1, 15, 30. Each blood specimen was processed according to standard cell processing and data acquisition protocols, and three endpoints were measured: percentage of reticulocytes, frequency of mutant phenotype reticulocytes, and frequency of mutant phenotype erythrocytes. Each laboratory observed dose-related increases in the frequency of mutant phenotype cells on Day 15, and the responses were main- tained through the latest timepoint studied (Day 90). Furthermore, the resulting data show a remarkable level of agreement among laboratories, both in terms of the kinetics of the responses, as well as the magnitude of the induced mutant frequencies. This data indicate that this mutation assay has a high level of robustness and is easily transferable across laboratories. This sets the stage for future inter-laboratory studies that will increase the number of mutagens and non-mutagens evaluated in order to systematically characterize assay performance.

The integration of genotoxicity endpoints into general toxicity studies is attrac- tive for several reasons, including the potential to reduce animal use and to provide comprehensive toxicity information that aids interpretation of genotoxicity results. This laboratory has developed automated scoring techniques for monitoring two blood-based genotoxicity endpoints, thereby making integration practical: flow cy- tometry procedures for scoring micronucleated reticulocyte frequency and gene mutation at the Pig-A locus. The ability to integrate these endpoints into a 28-day repeat dosing schedule was investigated using N-ethyl-N-nitrosourea, 7,12-di- methyl-1,2-benz[a]anthracene, benzo[a]pyrene, and N-methyl-N-nitrosourea. Wistar Han rats were treated on 28 consecutive days via oral gavage. Day -1, 15, 29 and 56 blood specimens were analyzed for Pig-A mutation with a dual staining method (SYTO 13 in combination with anti-CD59-PE) that facilitated mutant cell frequency measurements in both total erythrocytes and the reticulocyte sub- population. Day 4 and 29 blood specimens were evaluated for MN-RET frequency according to MicroFlow® Kit instructions. Significant increases in %MN-RET were observed for all chemicals on Days 4 and 29. Increased mutant phenotype cell frequencies were evident on Day 15 for all chemicals, with higher frequencies ob- served in the Day 29 specimens. This was particularly evident in reticulocytes. Persistence of the Pig-A response was demonstrated by elevated frequencies of mu- tant phenotype reticulocytes and erythrocytes in Day 56 samples. These results clearly support the integration of MN-RET and Pig-A mutation endpoints into toxicity studies, as they provide valuable complementary genotoxicity data with the added advantage of reducing animal usage.

**USE OF CUSTOM COMPARATIVE GENOMIC HYBRIDIZATION (CGH) MICROARRAYS TO EVALUATE THYMIDINE KINASE (TK) MUTANTS OF L5178Y MOUSE LYMPHOMA CELLS.**

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2-amino-6-N-hydroxyadenine (AHA) is one of the few chemicals that has been shown to induce primarily point mutations in a variety of cell types. Tk mutants arising after exposure of mouse lymphoma cells to AHA were mainly of the large colony type, although small colony mutants were also induced. Twelve large colony Tk mutants and 10 small colony Tk mutants were independently isolated for mole- cular analysis of chromosome 11: the Tk locus is located on the distal end of chro- mosome 11. Analysis of microsatellite loci along chromosome 11 for loss of het- erozogosity (LOH) showed some degree of LOH (particularly for the Tk gene) for a majority of Tk mutants. However, for most Tk mutants in the large and small colony mutants. A custom-made CGH array containing 13,000 chromosome 11 probes spaced at approximately 10,000 base intervals was used to further evaluate genome aberrations in these Tk mutants. The results showed that there were no obvious genome aberrations in chromosome 11 within the large colony mutants, while two small colony Tk mutants showed large deletions that included the Tk locus. These re- sults are consistent with the microsatellite analysis. The results from array CGH analysis also showed that three small colony Tk mutants with LOH along the entire chromosome 11 retained 2 copies of chromosome 11, suggesting that these mu- tants arose through mitotic non-disjunction. Thus, array CGH provides a rapid, high resolution molecular karyotype allowing the determination of mechanisms of mutation induction.
were treated with various concentrations (10-150 μg/ml) of 11 CSCs for 4 hours. All treatments with these CSCs resulted in an increase of both cytotoxicity and mutagenicity in a dose-dependent manner. Using the data evaluation criteria developed by the MLA Workgroup of the International Workshop for Genotoxicity Testing, the lowest concentration giving a positive response was between 40-70 μg/ml with a relative total growth (RTG) of 50-70%, while the highest concentration with acceptable cytotoxicity (> 10% RTG) was within 75-150 μg/ml. To elucidate the underlying mutagenic mechanism, we examined the loss of heterozygosity (LOH) at four microsatellite loci (Tk1, D11Mit36, D11Mit20 and D11Mit74) spanning the entire chromosome 11 for the mutants induced by CSCs. The mutational spectra from the CSC treatments were significantly different from those of the control. Compared to spontaneous mutants, the CSCs induced more mutants at Tk locus only and with chromosome damage extended to 30 centimorgan. These results suggest that CSCs are mutagenic in mouse lymphoma cells with a clastogenic mode-of-action.

**Evaluation of Publicly Available Mouse Lymphoma Assay Data Using Currently Accepted Standards to Establish a Curated Database.**

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The forward mutation assay at the thymidine kinase (tk) locus of L5178Y/TK-/-3.7.2C mouse lymphoma cells (MLA) is one of the widely used *in vitro* assays to identify genotoxic potential of test materials. Publicly available data from this and other genotoxicity assays, primarily collected in 1980s, have been used to develop structure-activity relationships (SAR) and to draw correlations to animal carcinogenicity findings with less than satisfactory outcomes. Given that there have been significant procedural and data-evaluation enhancements in recent years to MLA under the auspices of FITG, there is a compelling need to re-examine the validity of the conclusions in earlier studies to better inform SAR and other models. Accordingly, publicly available data from more than 400 MLA studies were re-evaluated. Data were examined against pre-defined criteria for the acceptance of background mutant frequency, cloning efficiency, positive control values, appropriate dose selection, and data consistency. This analysis revealed that more than 40% of the “Positive” cells in these studies did not meet the current global evaluation factor (GEF) criteria for a positive response. It should be noted that “Positive” determinations were made in this re-analysis if a response met the GEF criteria, even if the assay did not pass all acceptance criteria. Strict adherence to all of the current criteria would have resulted in a vast majority of studies being considered as unacceptable and therefore uninterpretable. Furthermore, the lack of colony sizing data precluded a judgment on practically all of the “Negative” calls resulting in these studies being classified as “Inconclusive” in this re-analysis. Overall, more than 50% of the studies reviewed were classified as “Inconclusive”. Thus, the use of an expertly reviewed and curated MLA database will likely improve the validity of the outcomes from predictive models such as SAR and correlations to animal carcinogenicity assays.

**Genotoxicity of 2, 6- and 3, 5-Dimethylaniline, and their Metabolites in Chinese Hamster Ovary Cells.**

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The monocyclic aromatic amines, 2,6-Dimethylaniline (2,6-DMA) and 3,5-Dimethylaniline (3,5-DMA) are used in the manufacture of pesticides, dyestuffs and pharmaceuticals. Although 2,6-DMA has been shown to be carcinogenic in rats and mutagenic in S. typhimurium, little is known about its mechanism of action and the genotoxicity of 3,5-DMA. We investigated the cytotoxicity and mutagenicity of these alkylanilines with particular emphasis on the roles of cytochrome P4501A2 (CYP1A2), arylsulfoxtransferase (AST) and N-acetyltransferase (NAT2) in their metabolic activation. We first examined cytotoxicity and mutation induced in the gpt gene of CHO AS52 cells by 2,6- and 3,5-DMA activated by a human liver S9 preparation, or their synthetic hydroxylamine and aminophenol metabolites. Cells exhibited a dose-dependent decreased cell survival and increased mutant fraction upon all treatments, but showed considerable variation in potency, with aminophenol metabolites having the highest potency and parent compounds the least. The predominant mutations were single base substitutions located at G:C sites under all exposure conditions. Next, to investigate whether AST and NAT2 contributed to phase II bioactivation of these alkylanilines, we employed the optimized Ames test or predicted from PAC analytical data using currently accepted standards to establish a curated database.

The Salmonella mutagenicity assay is an important tool used to assess the mutagenic potential of chemicals. Historically, the assay test battery typically used included Salmonella strains TA98, TA100, TA1535, TA1538 and TA1537. The current OECD Salmonella test battery has been modified and includes the use of Salmonella strain TA102 and/or E. coli strain WP2 uvrA and/or WP2 uvrA pKM101. These two/three strains were added because they are sensitive to oxidative DNA damage and detect chemicals that induce mutations via that mechanism. Occasionally there are compounds that are mutagenic in the E. coli WP2 uvrA pKM101 or WP2 pKM101 strains only but not in any of the Salmonella strains including TA102. Compounds with this mutagenic profile are typically treated as Ames positive however there has not been a recent update to determine if the E. coli strain adds sensitivity to the assay. The most recent evaluations were conducted by Watanabe et al., (1996, 1998a and 1998b). Their evaluations originally suggested that there were 16/79 compounds which were positive in E. coli WP2 pKM101 or WP2 uvrA pKM101. They concluded that both TA102 and E. coli could be included in the standard battery to increase detection of mutagenic compounds. However, a re-evaluation of the data for these compounds suggests by modern evaluation standards only 10 compounds were actually uniquely positive in E. coli. There are now data indicating that 6 of these compounds are positive in one of the Salmonella strains including TA102 and 9 are positive in at least one of the in vitro genetic toxicity assays. Several of the compounds that were positive in E. coli and one of the in vitro mammalian assays have been tested and shown to be rodent non-carcinogens. Based on this, compounds that are mutagenic in E. coli only but not mutagenic in any of the other Salmonella strains and are not genotoxic in one or more in vitro mammalian assays are unlikely to represent a genotoxicity or carcinogenicity hazard.

**Predicting the Outcome of Optimized Salmonella Assays.**

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Historically the petrochemical industry has used the optimized Ames test as a means of identifying certain petroleum streams which have the potential to cause dermal cancer via genotoxic processes. In essence this test is a biological indicator of a pattern of polycyclic aromatic constituents (PAC) present at levels that are toxicologically relevant. An analytical test was also developed in which the aromatic fractions of petroleum streams were separated by gas chromatography, providing information on the aromatic constituents by ring number. Subsequently a statistical model was developed in which 1-7 ring PAC contents of approximately 250 samples were assessed, their mutagenic potency was predicted, and the predicted values were compared to experimental data. Although the standard regression predicted well across the entire mutagenic potency spectrum (r=0.95), the predictions were poor below a mutagenic index score of 2, the relevant region for determining potential carcinogenicity. An iterative non-linear model that made a bivariate prediction of the optimized Ames mutagenic index (above or below the critical value of 1.0) was developed based on a training set of 196 of the original samples. The resulting model agreed with the experimentally determined results 98% of the time, and, when the remaining 50 “hold out” samples were tested, 96% of the predictions were in agreement with the experimentally determined values. It was concluded that among those petroleum streams for which the optimized Ames test is valid, i.e., streams with boiling ranges > 300oC, the mutagenic potential can be assessed directly using the optimized Ames test or predicted from PAC analytical data using the statistical model.
PRMTUGEN ACTIVATION AND P450 ACTIVITIES OF LIVER, KIDNEY, AND LUNG S9 FROM MALE SPRAGUE-DAWLEY RATS TREATED WITH P450 INDUCERS.

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Organ-specific genotoxicity is a known phenomenon which may be a result of organ-specific activation of promutagens. Promutagen activation is routinely studied using liver homogenate supernatant (S9 fraction) as an exogenous activating system. We report here our investigation on the use of S9 fractions from nonhepatic organs for the evaluation of organ-specific genotoxicity. Male Sprague-Dawley was treated with 75 mg/kg body weight of phenobarbital for 4 days (days 1 to 4) as well as 80 mg/kg body weight of β-naphthoflavone for two days (days 3 to 4). The animals were sacrificed on day 5 and S9 from liver, kidney and lung were prepared. The S9 fractions from the three organs were evaluated for promutagen activation potential in the Salmonella/histidine-reversion mutagenicity assay (Ames test; strains TA98, TA100, TA1535, and TA1537) and the Escherichia coli WP2uvrA assay. Results with the model promutagen 2-aminoanthracene show that S9 from all three organs had strong promutagen activating potential. Mutagenicity expressed as fold induction values over solvent control were 39 (liver), 35 (kidney), and 9 (lung) for TA98; 15 (liver), 15 (kidney), and 4 (lung) for TA100; 14 (liver), 10 (kidney), and 7 (lung) for TA1535; 47 (liver), 22 (kidney) and 8 (lung) for 1537; and 4 (liver), 3 (kidney) and 3 (lung) for WP2uvrA. The P450 activities of the S9 fractions were evaluated using 7-ethoxyresorufin (CYP1A) and luciferin-IPA (CYP3A) as substrates. CYP1A activity was found to be similar for liver and kidney S9, and approximately 50% less for lung S9. CYP3A activity was substantially higher for the liver S9 than for kidney and lung. The mutagenicity results of 2-aminoanthracene are therefore consistent with the known phenomenon that poly-cyclic hydrocarbons are mainly activated by CYP1A. The results showed that S9 from nonhepatic tissues are active in promutagen activation and may be used to aid the evaluation of organ-specific genotoxicity.

GENOTOXICITY STUDIES OF TACRINE.


Toxicology, Swen Life Sciences Limited, Hyderabad, Andhra Pradesh, India. Sponsor: V. Reddy.

Tacrine (1,2,3,4-Tetrahydro-9-acinidine / THA), a reversible cholinesterase inhibitor is being widely used for the treatment of mild to moderate dementia from Alzheimer’s disease. Available literature had shown that tacrine and its structurally related compounds could induce mutagenicity. Current study was planned to explore the mutagenicity in vivo and in vitro as well as a possible involvement of promutagen activation in vivo micronucleus in bone marrow of Wistar rats and in vitro reverse mutations (AMES) in salmonella TA strains (TA98, TA100, TA1535 and TA1537) with different metabolic activation by using rat liver S9 fraction. Highest concentration of tacrine tested along with concurrent controls was 1250 μg/ml/plate followed by five lower concentrations with 2-2.5 fold spacing. For micronucleus test 6–8 weeks old Wistar rats were divided into five groups of 5 rats per sex per group and were gavaged with 0, 5, 10 and 20 mg/kg Tacrine for 2 days and 1 mg/kg Mitomycin C as a positive control. Survived rats were sacrificed 24 hours after last dose, bone marrow aspirates were collected from femur joints and smears were prepared to determine the clastogenicity through P/E ratio and the incidence of MNCE. Salmonella Reverse mutation assay showed no mutagenicity among all strains tested except TA 1537 with or without S9 fraction. There was no evidence of clastogenicity in rat bone marrow micronucleus test up to 20 mg/kg Tacrine. In conclusion, tacrine could cause mutagenicity at framework target sequence in TA 1537 strain but not clastogenicity.

CONTRIBUTION OF SINGLE SMOKE CONSTITUENTS TO THE MUTAGENIC ACTIVITY OF THE GAS/VAPOR PHASE OF CIGARETTE MAINSTREAM SMOKE.

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The water soluble fraction of the gas/vapor phase (GVP) of 2R4F cigarette mainstream smoke has been shown to be mutagenic in Salmonella typhimurium strain TA100 without S9 metabolic activation in the microsuspension version of the Salmonella reverse mutation assay. To estimate the contribution of single smoke constituents to the mutagenic activity of GVP from the Reference Cigarette 2R4F, GVP was screened for smoke constituents by a targeted GC/MS screening method and an HPLC method (formaldehyde only). 65 single smoke constituents, from 13 chemical classes were identified, with the aldehydes having the highest yields. The ten constituents with the highest yields, i.e., acetaldehyde, acetone, 2,3-butanedione, 2-butanol, acetonitrile, acrolein, propionaldehyde, methyl vinyl ketone, crotonaldehyde, and toluene, as well as five other constituents which are reported to be mutagenic, i.e., methacrolein, formaldehyde, benzene, pyridine, and 2-furaldehyde, were assayed as pure substances for their mutagenic activity. Results showed that the mutagenic activity of GVP from the 2R4F could be attributed mainly to six smoke constituents: acrolein, crotonaldehyde, formaldehyde, methyl vinyl ketone, methacrolein, and 2,3-butanedione. Of these six constituents, acrolein, crotonaldehyde, and formaldehyde account for approximately 60% of the mutagenicity.

INDUCTION OF THE BASE EXCISION REPAIR GLYCOSYLASE NIEL1/2 IN ANILINE-INDUCED SPLENIC TOXICITY.

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The mechanisms by which aniline exposure elicits splenotoxic response, especially the tumorigenic response, are not well-understood. Earlier, we have shown that aniline-induced oxidative stress is associated with increased oxidative DNA damage in the spleen. Base excision repair (BER) pathway is the major mechanism for the repair of oxidative DNA base lesions, and we observed an up-regulation of 8-oxoguanine glycosylase 1 (OOG1), a specific DNA glycosylase involved in the removal of 8-OHdG adducts, following aniline exposure. Nei-like DNA glycosylases (NEIL1/2) belong to a family of BER proteins that is distinct from other glycosylases, including OOG1. This study was, therefore, focused on evaluating if NEILs have a role in the repair of oxidative DNA lesions in the spleen following aniline exposure. To achieve that, male SD rats were subchronically exposed to aniline (0.5 mmol/kg/day via drinking water for 30 days), while controls received drinking water only. The BER activity of NEIL1/2 was assayed using a bubble structure substrate containing 5-OHU (targeted by NEIL1 and NEIL2) and by quantitating the cleavage products. Aniline treatment led to a 1.2-fold increase in the NEIL1/2-associated BER activity in the nuclear extracts of spleen compared to the controls. Furthermore, Western blot analysis showed that protein expression of NEIL1 and NEIL2 in the nuclear extracts of spleen from aniline-treated rats was 2-3.8-fold higher than controls, respectively. Aniline treatment also led to stronger immunoreactivity for NEIL2 in the spleens, confined to the red pulp areas. These studies, thus, show that aniline-induced oxidative stress is associated with an induction of NEIL1/2. The increased BER activity of NEILs could be important in the removal of oxidative DNA lesions, especially in transcribed DNA, in the splenic toxicity of aniline. Supported by NIH ES06476.

XPC IS ESSENTIAL FOR NUCLEOTIDE EXCISION REPAIR OF ZIDOVUDINE-INDUCED DNA DAMAGE IN HUMAN HEPATOMA CELLS.


Zidovudine (3’-azido-3’-deoxythymidine, AZT), a nucleoside reverse transcriptase inhibitor, can be incorporated into DNA and cause DNA damage. The mechanisms underlying the repair of AZT-induced DNA damage are unknown. To determine whether or not the nucleotide excision repair (NER) pathway plays a role in AZT-induced DNA damage, human hepatoma HepG2 cells were incubated with AZT (0, 2, 20, or 100 μM) for 2 weeks and the expression of NER genes was then determined using a pathway-based real-time PCR array. AZT treatment significantly decreased the total viability of HepG2 cells and lactate dehydrogenase activity but increased caspase 3/7 activity when compared to nontransfected HepG2 cells or HepG2 cells transfected with a scrambled short hairpin RNA sequence. These data indicate that XPC may play an essential role in the repair of AZT-induced DNA damage. (Supported by Interagency Agreement 224-07-0007 between NCTR/FDA and NIEMHS/NTP).

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704 IDENTIFICATION OF A MOUSE NOVEL GENE THAT WAS INDUCED UPON GENOTOXIC STRESS AND POTENTIALLY INVOLVED IN DNA DAMAGE RESPONSE OR REPAIR.


Identifying genotoxic stress responsive genes and using these genes as molecular biomarkers is one of prospective approaches for genotoxicity assessment. In our previous microarray study performed in mice administrated with 7 genotoxic chemicals and 4 non-genotoxic chemicals, we identified a novel gene named as BC, whose expression was specifically up-regulated by most genotoxic chemicals, while remained nearly unchanged upon non-genotoxic chemicals. Some microarray data related to BC expression was further confirmed by Real-Time PCR. Our research aimed to examine the relationship between BC expression and genotoxic stress, elucidate molecular characteristics of BC and uncover its cellular function. Multiple genotoxic agents, such as methyl methanesulfonate (MMS), etoposide and γ-ray up-regulated BC mRNA expression in NIH3T3 cells. Expression of BC induced by MMS was in a dose-dependent manner and well paralleled with micronucleus formation. Time-course analysis indicated that BC began to be induced at 2 hr, peaked at 4 hr and backed to normal level at 8 hr after 80Gy γ-ray irradiation. Northern-blot and RACE (Rapid Amplification of cDNA Ends) results showed that BC gene had three main transcripts (approximate 2.7, 2.8 and 5.5 kb) consistent with the information in GenBank. Expression of BC could be detected in multiple mouse tissues including heart, liver, spleen, lung, kidney, testis and brain by Real-time PCR. Suppression of BC in NIH3T3 cells by RNA interference resulted in more severe DNA damage and chromosome damage, as shown by alkaline comet assay and micronucleus test, after γ-ray irradiation. Taken together, we identified a DNA damage response related novel gene (BC) which was induced upon genotoxic stress, and could be a potential biomarker for genotoxicity.

705 EFFECT OF DEFICIENT BASE EXCISION REPAIR (BER) STATUS ON METHYLmercury (MeHg)-INITIATED TOXICITY IN VITRO.

S. L. Ondov2, G. P. McCallum and P. G. Wells1,2. 

Dietary MeHg exposure can damage the adult and fetal central nervous system, potentially in part through production of reactive oxygen species (ROS) and oxidative DNA damage. To assess the roles of ROS and oxidative DNA damage, and the protective role of DNA repair, we used mouse embryonic fibroblasts (MEFs) from wild-type (WT) and oxoguanine glycosylase 1 (Ogg1) knockout (KO) mice, the latter deficient in repair of the oxidative lesion 8-oxo-2′-deoxyguanosine (8-oxodG). Following 6-hr incubation with 0-10 μM MeHg, a concentration-dependent decrease in cell viability was observed in all cells, with the Ogg1 KO MEFs appearing less viable at all concentrations, compared to WT. The decrease in viability was not reduced by a 30-min preincubation with the free radical spin trapping agent DMPO. As above, skin samples were collected post exposure and analyzed for PAR. Reduced PAR levels were detected in arsenite treated animals as compared to controls indicating inhibition of PARP-1 activity by arsenite. These results show that in vitro observations are retained in an in vivo setting and help to further the understanding surrounding interactions of arsenic and ultraviolet radiation in skin. This work was supported by NIH award R01 ES015826.

706 CHARACTERIZATION OF POLY(ADP-RIBOSE)POLYMERASE-1 KINETICS AND INHIBITION BY ARSENITE: AN IN VIVO STUDY.

B. S. King, K. L. Cooper and L. G. Hudson. 

Skin is a target tissue for arsenic carcinogenesis. Low arsenic concentrations enhance DNA damage and skin tumors in mice following ultraviolet radiation (UVR) exposure, but, the underlying mechanisms are unclear. Inhibition of DNA repair enzymes such as Poly(ADP-ribose)polymerase-1 (PARP-1) by arsenic are under investigation, yet little is known about PARP-1 activation by UVR in the skin. The initial goal was to characterize the in vivo kinetics of PARP-1 activation following UVR. Skh-1 (hairless) mice were exposed to a single dose of solar simulated UVR (28 kJ/m²). Skin samples were collected post exposure as well as from control animals and analyzed for poly(ADP-ribose) (PAR), a branched polymer attached to acceptor proteins by PARP-1, using immunohistochemistry. PARP-1 activation was found to be rapid and did not persist more than 4-6 hours post exposure. Using the same experimental design, the next objective was to observe PARP-1 inhibition by arsenic in vivo. Skh-1 mice were exposed to 5 mg/L sodium arsenite or non-treated water for 28 days then exposed to a single dose (28 kJ/m²) of solar simulated UVR. As above, skin samples were collected post exposure and analyzed for PAR. Reduced PAR levels was detected in arsenite treated animals as compared to controls indicating inhibition of PARP-1 activity by arsenite. These results show that in vivo observations are retained in an in vivo setting and help to further the understanding surrounding interactions of arsenic and ultraviolet radiation in skin. This work was supported by NIH award R01 ES015826.

707 IDENTIFYING GENOTOXIC COMPOUNDS USING A BATTERY OF ISOGENIC DNA REPAIR DEFICIENT DT40 CELL LINES IN A QUANTITATIVE HIGH-THROUGHPUT SCREENING (QHTS) PLATFORM.

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DNA repair pathways play a critical role in cellular homeostasis by repairing DNA damage induced by endogenous processes and xenobiotics. Isogenic chicken DT40 cell lines deficient in different DNA repair pathways can be used to identify genotoxic compounds and aid in characterizing the nature of the DNA damage induced. As part of the U.S. Tox21 program, we evaluated the activity of seven isogenic DNA repair deficient DT40 cell lines to identify direct-acting genotoxic chemicals within an NTP 1408 compound library. Evaluation was based on increased cytotoxicity in one or more of the cell lines deficient in a DNA repair pathway (e.g., pol β, ku70/70 rat αC, ubc13, rev3, fance) compared with the wild-type cell line. The assays were optimized for qHTS in a 1536-well plate format. We identified several well-known genotoxic compounds (e.g., melphalan, Adriamycin) as well as other compounds that induced differential cytotoxicity in one or more DNA repair deficient cell lines. Active compounds were evaluated further by comparing the frequency of induced chromosomal damage in the appropriate DNA repair deficient cell lines and the wild-type cell line. Our results demonstrate the utility of this approach for screening large compound libraries for genotoxic activity using 1536-well based qHTS and for acquiring detailed information on the type(s) of DNA damage induced by these compounds. Supported by NIEHS Interagency Agreement Y2-ES-7020-01.

708 METHANOL EXPOSURE DOES NOT LEAD TO ACCUMULATION OF OXIDATIVE DNA DAMAGE IN MICE, RABBITS, OR PRIMATES.

G. P. McCallum1, M. Sul1, S. L. Ondov1 and P. G. Wells1,2,3. 

In vitro and in vivo genotoxicity tests with methanol (MeOH) indicate that it is not a mutagen, but controversy exists regarding the carcinogenic potential of this agent due to conflicting reports in long-term rodent cancer bioassays. One potential mechanism whereby MeOH could indirectly damage DNA is via free radical-initiated, reactive oxygen species (ROS)-mediated oxidative DNA damage. To investigate this possibility we treated male CD-1 mice, New Zealand white rabbits, and cynomolgus monkeys with MeOH (2.0 g/kg ip) and at 6 hr assessed tissue oxidative DNA damage, measured as 8-oxo-2′-deoxyguanosine (8-oxodG) by HPLC with electrochemical detection. We found no MeOH-dependent increases in 8-
oxodG in bone marrow, spleen, kidney, lung or liver of any species. Chronic treatment of CD-1 mice with MeOH (2.0 g/kg ip) daily for 15 days also did not increase 8-oxodG levels in these organs. Further studies in DNA repair-deficient oxoguanine glycosylase 1 (Ogg1) knockout (KO) mice support these findings. Fibroblasts from Ogg1 KO mice accumulated 8-oxodG at 6 h following acute exposure to the ROS-initiating renal carcinogen potassium bromate (KBrO3; 2.0 mM), but did not accumulate 8-oxodG following exposure to 125 mM MeOH. In vivo exposure of Ogg1 KO mice to KBrO3 (100 mg/kg ip) doubled renal 8-oxodG levels 24 h post-dose. In contrast, MeOH (2.0 g/kg ip) did not alter renal levels of 8-oxodG at 6 or 24 h post-dose in Ogg1 KO mice. Taken together these observations suggest that MeOH exposure does not promote the accumulation of oxidative DNA damage, and that it is unlikely that environmental exposure to MeOH would lead to carcinogenesis via this mechanism. (Support: Methanol Foundation, CIHR, CHIR Research and Development postdoctoral award [GPM], CHIR Frederick Banting and Charles Best Doctoral Award [SLO]).

709 POTENT INHIBITION OF PEROXYNITRITE-INDUCED DNA STRAND BREAKAGE AND HYDROXYL RADICAL FORMATION BY THE COMMONLY USED DRUG VEHICLE DMSO AT EXTREMELY LOW CONCENTRATIONS.

Z. Isa, H. Zhu, Y. Li and H. P. Miau. Edward via Virginia College of Osteopathic Medicine, Virginia Tech Corporate Research Center, Blacksburg, VA.

Dimethyl sulfoxide (DMSO) is frequently used as a solvent for many water-insoluble drugs in biological studies at concentrations often up to 1%. However, little is known about its effects on oxidative DNA damage at very low concentrations (0.005-0.5%). This study was undertaken to investigate the effects of DMSO on peroxynitrite-induced DNA strand breaks, a critical event leading to peroxynitrite-elicted cytotoxicity. Incubation of Xφ-174 plasmid DNA, with 3-morpholinosydnonimine (SIN-1), a peroxynitrite generator, led to the formation of DNA strand breaks in a concentration and time-dependent fashion. The presence of DMSO at concentrations of 0.005-0.5% was found to significantly inhibit SIN-1-induced DNA strand breaks in a concentration-dependent manner. However, DMSO at the above concentrations showed no effect on SIN-1-mediated oxygen consumption, indicating that DMSO did not affect the auto-oxidation of SIN-1 to form peroxynitrite. It is observed that incubation of the plasmid DNA with authentic peroxynitrite resulted in significant formation of DNA strand breaks, which could also be dramatically inhibited by the presence of DMSO at 0.005-0.5% EPR spectroscopy, using 5,5-dimethylpyrroline-N-oxide (DMPO) as a spin trap demonstrated the formation of DMPO-hydroxyl radical adduct (DMPO-OH) from the SIN-1 and authentic peroxynitrite. DMSO at the concentrations ranging from 0.01% to 0.5% significantly inhibited the adduct signal. Taken together, these studies demonstrate, for the first time, that DMSO at extremely low concentrations (0.005-0.5%) can potently inhibit peroxynitrite-mediated DNA strand breakage and hydroxyl radical formation. The results of this study suggest that, where DMSO is applied as a solvent, caution should be observed when evaluating the actions of drugs in experiments involving DNA damage.

710 IDENTIFICATION OF A NOVEL ROLE FOR THE RNA SURVEILLANCE PROTEIN, UPF1, IN OXIDATIVE STRESS INDUCED P53 ACTIVATION.


Genotoxic stress is known to activate the phosphatidylinositol-3-kinase-like kinases (PIKKs); ATM, ATR, and SMG1 to phosphorylate the tumor suppressor protein p53, a protein central to coordination of cell cycle arrest and apoptosis. In addition to its role in monitoring DNA damage, SMG1 plays an important role in the RNA surveillance pathway as a nonsense mediated mRNA decay (NMD). During NMD SMG1 phosphorylates Upf1, leading to the recruitment of decay proteins to eliminate misspliced RNAs. Using hyperoxia as a model to induce chronic oxidative stress and DNA damage, we now show that Upf1 is also necessary for full activation of p53, suggesting further interplay between RNA and DNA surveillance pathways. siRNA knock down of Upf1 protein before exposure to hyperoxia decreased the phosphorylation and accumulation of p53 in three independent cell lines. Upf1 mediated changes were post-translational and not involving mdm2, a master negative regulator of p53. Although Upf1 knock down has previously been shown to stall cell cycle, which could indirectly affect p53 activation, loss of Upf1 did not prevent cell cycle progression. Our studies suggest two possible mechanisms. Upf1 could be involved in response to both damaged DNA as a mediator of p53 activation and RNA in NMD. On the contrary, damaged RNA could activate pathways that up until this point have been thought to respond wholly to DNA damage, and this could be mediated through Upf1.

711 EXPLORING MECHANISMS OF INFLAMMATION-ASSOCIATED SYSTEMIC GENOTOXICITY.

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We have previously demonstrated acute and chronic intestinal inflammation characteristic to inflammatory bowel disease patients induces genotoxicity not only locally in the colon, but also systemically in the peripheral blood, utilizing several mouse models of intestinal inflammation. We believe this may be a mechanism for inflammation-associated carcinogenesis for cancers arising outside the site of inflammation, such as lymphoma. In order to further characterize the resultant systemic genotoxicity and explore causative mechanisms, we identified and characterized genotoxicity in other cell types and subpopulations of cells, measured correlation of disease activity to the observed genotoxicity, and determined the role of cytokines in inducing systemic genotoxicity. Utilizing the alkaline comet assay and gel shift assay in human mononuclear cells, we observed disease activity to correlate to cytokines TNF-α and IL-10, which correlated to increased genotoxicity in all cell types tested. In addition, we observed the generation of DNA strand breaks, a critical event leading to peroxynitrite-mediated DNA strand breakage, more efficiently in CD4+ and CD8+ T cells versus other cell types in the peripheral blood, and also detected in mesenteric lymph nodes, peripheral lymph nodes, and in the intestinal epithelial cells. Levels of DNA damage also correlated to severity of clinical inflammation, in dextran sodium sulfate treated mice and in IL-10 deficient mice. Injection of the cytokine TNF-α into wild type mice was sufficient to induce genotoxicity, and levels of DNA damage were sustained for longer period of time when combined with injection with IL-1β. In conclusion, systemic DNA damage is characteristic to multiple organs and cell types, correlates to disease activity, and TNF-α is sufficient to induce the observed inflammation-associated systemic genotoxicity.

712 DOUBLE STRAND BREAK REPAIR IN HUMAN MITOCONDRIAL EXTRACTS.

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Mitochondrial DNA (mtDNA) is prone to double strand breaks (DSBs); however the nature of its repair in human cells is not understood. DSB repair was assayed in highly purified human mitoplasts using a newly developed quantitative PCR-based assay that measures ligation across a restriction site. Using this assay, we demonstrate DSB repair carried out by mitochondrial protein extracts consisting of only mitochondrial proteomes. DNA damage was highest in CD4+ and CD8+ T cells repaired more efficiently than blunt-ended DNA (13%, 8% and 3% repaired, respectively). Further investigation of the rejoining of PstI-generated DSBs revealed appreciable DNA processing, resulting in the loss of approximately 50 bases surrounding the PstI site. Sequence analysis of over 100 ligation products revealed several patterns of repaired DNA, most with deletions spanning 4-7 bp direct repeats. Results indicate that broken DNA is resected to reveal regions of microhomology, thus allowing annealing and ligation; producing DSB repair-mediated DNA deletions. These observed deletions are strikingly similar to those seen in patients of mitochondrial disorders such as progressive external ophthalmoplegia, Kear-Sayre syndrome and Pearson Syndrome. We have determined that the exonuclease function of DNA polymerase gamma mediates the 3’ to 5’ resection and is required for the efficient repair of DSBs. Additionally, we hypothesize that the 5’ to 3’ exonuclease responsible for the resection is hSNM1B. hSNM1B has a predicted mitochondrial targeting sequence with a putative cleavage site of 70 amino acids. Using immunofluorescence, hSNM1B-GFP is shown to be targeted to mitochondria. Furthermore, hSNM1B is more expressed in the mitochondrial matrix proteins. DNA containing 5’ or 3’ overhangs is repaired more efficiently than blunt-ended DNA (13%, 8% and 3% repaired, respectively). We have previously demonstrated acute and chronic intestinal inflammation characteristic to inflammatory bowel disease patients induces genotoxicity not only locally in the colon, but also systemically in the peripheral blood, utilizing several mouse models of intestinal inflammation. We believe this may be a mechanism for inflammation-associated carcinogenesis for cancers arising outside the site of inflammation, such as lymphoma. In order to further characterize the resultant systemic genotoxicity and explore causative mechanisms, we identified and characterized genotoxicity in other cell types and subpopulations of cells, measured correlation of disease activity to the observed genotoxicity, and determined the role of cytokines in inducing systemic genotoxicity. Utilizing the alkaline comet assay and gel shift assay in human mononuclear cells, we observed disease activity to correlate to cytokines TNF-α and IL-10, which correlated to increased genotoxicity in all cell types tested. In addition, we observed the generation of DNA strand breaks, a critical event leading to peroxynitrite-mediated DNA strand breakage, more efficiently in CD4+ and CD8+ T cells versus other cell types in the peripheral blood, and also detected in mesenteric lymph nodes, peripheral lymph nodes, and in the intestinal epithelial cells. Levels of DNA damage also correlated to severity of clinical inflammation, in dextran sodium sulfate treated mice and in IL-10 deficient mice. Injection of the cytokine TNF-α into wild type mice was sufficient to induce genotoxicity, and levels of DNA damage were sustained for longer period of time when combined with injection with IL-1β. In conclusion, systemic DNA damage is characteristic to multiple organs and cell types, correlates to disease activity, and TNF-α is sufficient to induce the observed inflammation-associated systemic genotoxicity.
young or adult male rats (gpt-delta transgenic F344 rats 3w, 11w or SD rats 3w,11w) with 20-80 ppm or 50-200 ppm of AA in drinking water for 28 days, and examined the genotoxicity in the blood, liver, testis. We also analyzed DNA adducts (N7-Ga-Gua) derived from GA in the liver, testis, mammary gland and thyroid gland. We observed the dose-related increases of micronuclei in peripheral blood. In liver, alkaline comet assay showed positive results in the middle and high doses, but the gpt mutations were not induced. In contrast, AA in peripheral blood and liver were not severe in these experiments, and did not observe significant difference between the young and adult rats. In contrast, AA caused significant genotoxic response in the micronuclei, comet and the gpt mutations. DNA adduct analysis showed that N7-Ga-Gua was significantly increased in tests and mammary gland in a dose-dependent manner. The adduct level of tests in the high dose was 8-folds higher in young rats than in adult rats. The genotoxicity of AA in peripheral blood and liver were not severe in these experiments, and did not observe significant difference between the young and adult rats. In contrast, AA caused significant genotoxic response in the micronuclei, comet and the gpt mutations.

We may be concerned about germinal mutagenicity and reproductive toxicity of children exposed to AA through ordinary foods.

714 PREGNANE X RECEPTOR (PXR) PROTECTS LIVER CELLS AGAINST DNA DAMAGES: EVIDENCE AND MECHANISMS.

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Pregnane x receptor (PXR) is a nuclear receptor that plays an important role in the pharmacokinetics of a broad spectrum of endogenous and xenobiotic compounds through coordinated-regulated transcriptional expression of both phase I, II metabolizing enzymes, as well as phase III transporters. In the early study, using HepG2 with stable-transfection of PXR, we found that PXR plays a role in reducing Benzo[a]pyrene (BaP)-induced DNA damage as determined by P32-postlabeling experiment. One mechanism for the PXR-mediated reduction of BaP-induced DNA damage is through metabolic detoxification through PXR-regulated detoxification system as reported earlier (Toxic. Sci. 104,67). A second mechanism that PXR activation causes trans-activation of the aryl hydrocarbon receptor (AhR) which is required for the BaP bioactivation. In current study, we discovered new evidence suggesting PXR plays a role in DNA repair: using Comet assay, we found UV irradiation-induced DNA damage was repaired significantly faster in HepG2 cells overexpressed PXR in comparison with parental HepG2 cells. Using Western blot analysis, UV-irradiation-induced H2Ae phosphorylation was significantly reduced in PXR-HeptG2 cells. In a recent study, we demonstrated PXR regulates nuclear translocation and substrate specificity of protein arginine methyltransferase PRMT1 (JBC 284, 9199). PRMT1 can methylate MRE11, a key enzyme in DNA double-strand break repair and genome stability. Taken together, these results suggest that in addition to metabolic detoxification and PXR-AhR crosstalk, PXR protects cells against BaP-induced damage through regulating DNA repair machinery. This research is supported by ES09859 and ES09106.

715 ASSESSING THE DNA DAMAGE POTENTIAL OF CHEMICALS VIA ACTIVATION OF THE p53 PATHWAY USING QUANTITATIVE HIGH-THROUGHPUT SCREENING (qHTS).

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Identifying compounds with potential to induce DNA damage is an important component of toxicological profiling. Compounds that induce DNA damage in mammalian cells often result in up-regulation of the tumor suppressor protein p53, a transcription factor critical to cell cycle regulation and activation of DNA repair processes. As part of the U.S. Tox21 program, we screened an NTP 1408 compound library that activated the p53-C35-signaling pathway in immortalized mouse embryo fibroblast (3T3-L1) cell line using a qHTS platform. The assay utilized a FRET substrate providing two-color readout and HCT-116 (human colon carcinoma, p53+/−) cells containing a stably integrated beta-lactamase reporter gene under control of the p53 response element (Invitrogen) shown to respond to known genotoxic compounds (e.g., mitomycin C, etoposide). After a 16-hr compound treatment, fluorescence intensity was measured at 460 and 530 nm. Each data point was expressed as the ratio of 460 nm/530 nm emissions to minimize well-to-well variation. Fourteen-point dose response curves were generated for each compound; ~130 potentially active compounds were identified, including several known DNA-reactive compounds (e.g., melphalan, 5-azacytidine, 2-aminoanthraheine). To confirm p53-dependency, a subgroup of compounds was counter-screened with the same HCT-116 reporter line after p53 deletion by siRNA silencing. Although most compounds were confirmed as p53-dependent, a few were active in the absence of p53, suggesting, among other possibilities, that they may activate other p53 family members, such as p73 and/or p63. Our results demonstrate the value of this approach as one component in a comprehensive qHTS test battery designed to screen large compound libraries for the presence of genotoxic compounds. Supported by NIEHS IAA Y2-ES-7020-01.

716 THE ROLE OF MYELOPEROXIDASE (MPO) IN DNA DAMAGE INDUCTION BY PCB3 METABOLITES.

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PCB3 has been detected in indoor and outdoor air. It is readily metabolized to dihydroxyquinone and quinones. In Chinese Hamster V79 cells, the PCB3 hydroydroquinone (PCB3-HQ) produced chromosome loss, whereas the para-quinone (PCB3-pQ) very efficiently induced gene mutations and chromosome breaks. We hypothesized that the genotoxicity of PCB3-HQ requires enzymatic activation by peroxidases, whereas the PCB3-pQ reacts directly with DNA and/or proteins. Employing human promyelocytic leukemia (HL-60, MPO-rich) and T-cell leukemia (Jurkat, MPO-poor) cell lines, we measured DNA damage (Comet assay, oxidative damage with FLARE), intracellular levels of reactive oxygen species (ROS), free −SH groups, and GSH after exposure to these PCB3 metabolites. We also examined the effects of normal/low temperature, MPO reduction (sucinylation of SGlutathione (S-GSH)), or GSH depletion in HL-60 cells on these parameters. PCB3-pQ increased intracellular ROS levels and induced DNA damage in both, HL-60 and Jurkat cells at 37°C and 6°C. MPO-reduction had no effect. PCB3-pQ also reduced intracellular free −SH groups and GSH in normal and GSH-depleted cells. In contrast, PCB3-HQ had no effect on GSH in HL-60 cells and reduced free −SH groups only at the highest concentration in GSH-depleted cells. Moreover, PCB3-HQ induced DNA damage and ROS production only at 37°C in HL-60 cells; no significant DNA damage and ROS increase was observed in Jurkat cells or HL-60 cells at 6°C or after MPO-reduction. These studies show that the effect of PCB3-HQ is enzyme dependent, i.e., PCB3-HQ is oxidized by MPO in HL-60 cells with the production of ROS and induction of DNA damage. However, PCB3-pQ may produce DNA damage by direct interaction with DNA or nuclear proteins like topoisomerase II, or possibly by indirectly increasing intracellular ROS levels by GSH depletion. The implication of metabolic activation of PCBs by MPO is novel and an important indication that PCBs may have tissue-specific cancer initiating activity. Supported by NIEHS R22 ES013661, ES05605 and DAMD17-02-1-2041.

717 INORGANIC CALCIUM (Ca2+) INDUCES DNA DAMAGE IN VIVO.

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Our current research has focussed on the toxicological implications of manipulating intracellular Ca2+ with the most recent and remarkable finding being the potential of inorganic calcium to induce DNA damage in vivo. The hypothesis put forward here is that by allosterically modulating the calcium sensing receptor (CaSR) present on the cell surface of the cells in the rat stomach, high doses of calcium carbonate cause a constitutive activation of the receptor due to the high local concentration in the stomach. The intracellular signalling pathway is thus over-activated, causing an extreme rise in intracellular calcium concentration and possibly other signalling pathways ultimately leading to DNA strand breaks. Several publications demonstrate that disruption of the content and movement of various calcium dependent Ca2+, can lead to inhibition of DNA synthesis, production of reactive oxygen species, DNA damage and ultimately apoptosis (Dopp et al. 2004). A clear link between activation of the CaSR and DNA strand breaks has been shown by Wu et al. in vitro by “rescue” of cells without a functional CaSR (Wu et al. 2005). In order to investigate this possibility, stomach cells harvested from rats dosed with 10 mg/kg/day for 3 days were assessed for statistical changes to both Tail Intensity and Moment relative to vehicle/control in Comet assays. A significant increase in both Tail Intensity and Moment over a 40 fold concentration range (50-2000 mg/kg/day) was observed after three doses at 0, 24 and 45 hours with necropsy at 48 hours. There was no evidence of histopathological changes in the stomach tissue or formation of reactive oxygen species. These results suggest that care should be taken when interpreting comet data from experiments using high doses of Ca2+-regulating agents that act on Ca2+-dependent signal transduction. Furthermore, studies with calcimimetic compounds are planned to confirm the proposed molecular action in vivo.
718 GENOTOXICITY OF ORGANIC EXTRACTS FROM THE AIR PARTICLES MEASURED IN AN ACELULAR SYSTEM WITH A NATIVE DNA.

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This study used the acellular model consisting of calf-thymus DNA eS9 (rat liver microsomal fraction) to assess the genotoxicity of complex mixtures of organic air pollutants adsorbed onto particles of various diameter in the air by means of DNA adduct analysis. We compared genotoxicity of the organic extracts (EOM) from dust particles of different aerodynamic diameter (0.17–10 μm) collected by high volume cascade impactors in 4 localities of the Czech Republic differing by the extent of the environmental pollution. For this purpose, we analyzed DNA adduct forming activity of extractable organic matter (EOM) from the particles in an acellular assay of calf thymus DNA coupled with 32P-postlabelling. Main findings of the study indicate that most of the observed genotoxicity is connected with fine particles (<1 μm). The hypothesis that B[a]P and carcinogenic PAHs (c-PAHs) contents in EOMs are the most important factors for their genotoxic potential was confirmed. The concentrations of c-PAHs in EOMs from the individual fractions indicate that fine fraction of 0.5–1 μm bounds highest quantity of c-PAHs in all localities. What is related to this, it may be the mass of the fraction that increases in all samples. Taking into account the relative mass of the specific size fraction, finest fraction of 0.17–0.5 μm is the most effective carrier of c-PAHs. Similarly, the DNA adduct levels are higher for the fraction of 0.5–1 μm in case that the adduct levels are normalized per m³ of the air, while the fraction of 0.17–0.5 μm revealed highest DNA adduct levels in case that PM quantities are taken into the consideration. Significant correlation was found between the concentrations of c-PAHs and DNA adduct levels induced in native DNA by EOMs from all the localities and various size fractions (R² > 0.98; p<0.001). Supported by the Czech Ministry of the Environment (grant #SP11/149/008).

719 DETERMINATION OF CYCLOSPORINE A IN RABBIT OCULAR TISSUES USING LC-MS/MS.

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Cyclosporine A is an immunosuppressant widely used following organ transplantation. It is also used in eye drops for the treatment of dry eyes. However, there is no sufficient information about ocular tissue distribution of cyclosporine A after application of eye drops due to lack of reliable assay method. The purpose of this work was to develop a reproducible method for the measurement of cyclosporine A in various ocular tissues which is related to the induction of fine fraction of 0.5–1 μm in the fraction. Taking into account the relative mass of the specific size fraction, finest fraction of 0.17–0.5 μm is the most effective carrier of c-PAHs. Similarly, the DNA adduct levels are higher for the fraction of 0.5–1 μm in case that the adduct levels are normalized per m³ of the air, while the fraction of 0.17–0.5 μm revealed highest DNA adduct levels in case that PM quantities are taken into the consideration. Significant correlation was found between the concentrations of c-PAHs and DNA adduct levels induced in native DNA by EOMs from all the localities and various size fractions (R² > 0.98; p<0.001). Supported by the Czech Ministry of the Environment (grant #SP11/149/008).

720 URINARY METABONOMIC ASSESSMENT OF DRUG-INDUCED PHOSPHOLIPIDOSIS (PLD) IN THE RAT.

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Mechanistic understanding and biomarker identification for PLD, a significant drug-development issue, has proven elusive. As part of a larger study, male rats were dosed for 29 days with either fluoxetine (30 mg/kg reduced to 15 mg/kg) or a trifluoromethyl tricyclic indole (TTI) compound (5 and 50 mg/kg), both previously characterized as PLD inducers. Alveolar macrophages collected on Day 29 and urine collected on Day 8 were used for targeted phospholipid (PL) analysis using LC/MS. Urine collected on Days 3, 8, 15, 22, and 29 and 3, 8, 22 and 29 was analyzed for global metabolonomic changes using NMR. Though differing in distribution, pathology data revealed PLD evident as early as Day 3 and fully developed by Day 8 for both compounds. NMR metabolomic analysis suggested that drug-induced changes were maximal by Days 3 and 8 and subsequently declined. Phenylacetylglycine, a proposed urinary biomarker of PLD, followed this transient trajectory. Assessment of numerous PLs (100) across several classes revealed marked increases in many individual PLs in urine (up to 50-fold) while some remained unchanged. Changes were dose-related with 50 mg/kg/day TTI producing increased (2-fold) phosphatidylcholines (PC) while fluoxetine produced elevated PCs (5 fold) followed by phosphatidylethanolamines (PE), phosphatidylinositol (PI) and ceramides with little effect noted on phosphatidylglycerols (PG) and phosphatidic acids (PA). In contrast, alveolar macrophage extracts from Day 29 drug-treated animals had little difference in PC, ceramide, PA, or PI while PS and PG and PI were elevated (2-3 fold) relative to control. These data suggest drug-induced changes in urinary peripheral biomarkers may be transient, occurring much earlier than previously anticipated. Additionally, PL profiles may help identify biomarker(s) of PLD and provide mechanistic insights into the etiology of this troublesome finding.

721 PRECLINICAL TOXICOLOGICAL ASSESSMENT OF SHET2A, A NOVEL CHEMOPREVENTIVE AGENT, IN RATS AND DOGS.

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The toxicity of SHetA2 (0, 25, 50, 100 and 500 mg/kg/day) was studied in CD® rats following 14 days of daily gavage administration. No drug-related mortalities and clinical signs of toxicity were seen in any dose groups. There were no significant changes in body weights, food consumption, clinical chemistry, hematology, coagulation tests, and relative organ weights. No treatment-related histopathological findings were seen in this study. There were no significant differences in total, oxidized and reduced glutathione liver levels (enzymatic recycling method; OxisResearch™ Glutathione assay kit). There were no significant differences in cardiac troponin T (cTnT), cardiac troponin I (cTnI), myosin light chain 3 (Myl3) and fatty acid binding protein 3 (FABP3) cardiotoxicity biomarker levels between the groups administered SHetA2 and the control group (Msd Cardiac Injury Panel 3 (rat) Assay Kit). Exposure was confirmed by LC-MS/MS determination of plasma and drug level of SHetA2 and its metabolites. SHetA2 plasma and liver levels were dose-dependently increased in the groups receiving the test article. SHetA2 levels appeared to be higher in the female rats. The no-observed adverse effect level (NOAEL) was not established and was considered above 500 mg/kg/day. The toxicity of SHetA2 was also assessed in male and female dogs following 14 days oral treatment by capsule/gavage administration at doses of 0, 100, 400 and 1500 mg/kg/day. Toxicity was noted only in as any dose group. There were no clinical signs of toxicity, decreases in body weights, and food consumption. No biologically significant changes in clinical chemistry, hematology, coagulation tests and urinalyses were seen in any SHetA2-treated groups. NOAEL was not determined in this study and was considered above 1500 mg/kg/day.

722 GENOME-WIDE SCREENING FOR DETERMINANTS OF ADRIAMYCIN SENSITIVITY IN SACCHAROMYCES CEREVIAE.

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Adriamycin is an anthracycline drug that is widely used in the treatment of various malignant tumors. However, acquisition of resistance to adriamycin by tumour cells and the development of adverse effects are disadvantageous in its clinical use. The mechanism underlying adriamycin toxicity is still not fully understood. To elucidate the detailed mechanism of adriamycin action, performed a genome-wide screening using a yeast deletion mutant collection (EUROSCARF). We found that disruption of several genes for endocytosis pathway and ubiquitin-proteasome system conferred resistance to adriamycin and for protein phosphatases, ribosomal proteins and cell signaling proteins conferred hypersensitivity to adriamycin in yeast cells. Interestingly, the adriamycin-resistant mutants, such as yeast cells lacking genes for endocytosis pathway or ubiquitin-proteasome system, were also resistant to other anthracycline drugs (daunorubicin and epirubicin), but not to bleomycin, cisplatin and 5-fluorouracil, suggesting that these pathway might specifically enhance sensitivity of yeast to adriamycin drugs. Most of the proteins
that we identified have not previously been reported to be associated to the adriamycin toxicity. A detailed further investigation on the relationship between these factors and adriamycin toxicity might be helpful for improvements in the chemotherapy with adriamycin.

723 ROLE OF PROTEIN PHOSPHATASE TYPE 1 (PP1) IN THE PROTECTIVE MECHANISM AGAINST TOXICITY OF ADRIAMYCIN.
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Adriamycin is an anthracycline antibiotic that is widely used in the treatment of leukemia and carcinomas. However, the detailed mechanisms responsible for the adriamycin toxicity remain unclear. To elucidate more detail mechanism of adriamycin toxicity, we have previously performed a genome-wide screen for determinants of adriamycin-sensitivity using budding yeast as a model eukaryote, and found that disruption of Reg1, a regulatory subunit of protein phosphatase 1 (PP1), increased sensitivity of yeast cells to adriamycin. Yeast PP1 is a heterodimeric complex composed of a catalytic subunit, Glc7, and multiple regulatory subunits. To clarify relationship between PP1 complex and adriamycin toxicity, we examined sensitivity of yeast cells lacking one of other regulatory subunits. Disruption of regulatory subunits, except for Reg1, did not affect sensitivity of yeast cells to adriamycin. Moreover, repression of expression of Glc7 sensitized yeast cells to adriamycin. We also found that the reg1 mutant yeast cell with a disruption of Glc7-binding site, which is required for phosphate activity, is hypersensitive to adriamycin. These results suggest that the phosphate activity of Glc7/Reg1 complex involved in protection against toxicity of adriamycin. On the other hand, tau-tomycin, a PP1 specific inhibitor, enhanced adriamycin toxicity in human breast MCF7 cells, suggesting that PP1 might be required for protection against adriamycin toxicity not only in yeast cells but also in human cells.

724 EFFICACY AND SAFETY STUDIES ON NOVEL, NQO1-DIRECTED LAVENDAMYCIN ANTI-CANCER AGENTS.
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Lavendamycin is a bacterially derived quinolinedione that displays significant antimicrobial and antitumor activities. NAD(P)H:quinone oxidoreductase 1 (NQO1) is a two-electron reductase implicated in the bioactivation of antitumor quinones. Expressed at high levels in many human solid tumors, NQO1 could potentially activate analogues of lavendamycin to exhibit selective toxicity to those tumors. We studied a series of novel lavendamycins substituted at the quinoline-7- and indolopyridine-2-positions, sites previously determined by computational and structure-activity studies to be important determinants of NQO1 substrate efficiency. Metabolism (reduction rates) of the quinones by purified recombinant hNQO1 was determined by a spectrophotometric assay that used cytochrome c as the terminal electron acceptor. Cytotoxicity was determined by a colorimetric assay. The best substrates for NQO1 were also the most selective toxic to human colon adenocarcinoma cells with high NQO1 activity (BE-NQ) versus those with no detectable NQO1 activity (BE-WT). The highest reduction rate by recombinant hNQO1 belonged to demethyllavendamycin morpholine amide at 103 +/- 8 μmol/min/mg. It also exhibited the greatest differential toxicity (9-fold) to the NQO1 expressing BE-NQ cells vs. the NQO1-null BE-WT cells. To assess safety to non-cancer cells, we cultured primary human aortic endothelial cells (HAECs), normal cells that are known to express appreciable levels of NQO1. In fact, the levels of NQO1 in HAECs (268 nmol/min/mg) were comparable with the BE-NQ cells (337 nmol/min/mg). Lavendamycin anologue, 7-N-acetyldemethyllavendamycin n-butylic amide, was nearly seven times less toxic to the HAECs than to the BE-NQ cells suggesting that NQO1 expressing normal cells may be less susceptible to lavendamycin toxicity than cancer cells. Supported by NIH Grant P20RR017670.

725 SILYMARIN SUPPLEMENTATION INCREASES GLUCONEOGENESIS PPAR-GAMMA AND PEPCK AND REDUCES LACTATE PRODUCTION IN LOW-INTENSITY EXERCISE RATS.
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The aim of present study was to investigate the effects of a flavonoid, silymarin (SM) supplementation, on serum levels (glucose, triglyceride and lactate) and the gluconeogenesis metabolism of the liver and muscle during low-intensity exercise in rats. Low intensity exercise was performed consisting of treadmill running at 8 m/min and 0-degree inclination for 60 min/day, 5 days/week, during 4 weeks. SM (50 mg/kg) and (-) epigallocatechin-gallate (EGCG 50 mg/kg, positive control) were orally administered. Serum was collected before and after exercise. At the end of experiment periods, the liver and skeletal muscle were (soleus, gastrocnemius) were quickly removed, weighed and stored at -80°C. Overall, the levels of triglyceride and lactate were decreased after low intensity exercise. Silymarin further reduced the levels of triglyceride and lactate. The levels of glucose were not altered after low intensity exercise and SM treatment. In the liver, mRNA level indicated the up-regulation of peroxisome proliferator activated receptor gamma (PPAR-gamma) and phosphoenol-pyruvate carboxykinase (PEPCK) after exercise and further increase in SM treated groups. In the skeletal muscle, phosphorylation of the pyruvate dehydrogenase kinase 4 (PDK-4) was increased after exercise and further increase in SM treated groups. Exercise induced the phosphorylation of 5′-AMP activated protein kinase (AMPK). However SM decreased the exercise-induced phosphorylation of AMPK. Taken together, SM supplementation enhanced low-intensity exercise-induced gluconeogenesis and beta-oxidation of fatty acid. These results suggest that a sufficient intake of silymarin, in combination with exercise, might improve endurance by modulating lipid and glucose metabolism, and could potentially decreased the development of lactate production and associated lifestyle-related diseases.

726 CHRYsin INHIBITS IGE-MEDIATED ALLERGIC REACTIONS AND INFLAMMATORY CYTOKINE PRODUCTIONS IN HUMAN MAST CELLS.
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A great number of people are suffering from allergic inflammatory disease such as asthma, atopic dermatitis, and sinusitis. Hence discovery of drugs for the treatment of these diseases is an important subject in human health. In this study, we investigated anti-allergic inflammatory effect of chrysin, on mast cell-based allergy model. Chrysin (5,7-dihydroxyflavone) is a natural flavonoid extracted from many plants. Several previous studies reported that it has various biological activities, such as anti-inflammation, anti-tumor, and anti-oxidant effects. Chrysin inhibited compound 48/80-induced systemic allergic reaction and serum histamine release. In addition, chrysin attenuates immunoglobulin E (IgE)-mediated passive cutaneous anaphylaxis (PCA) reactions. These inhibitory effects of chrysin were better than cromolyn, known as mast cell stabilizer. Gene expression of histamine H1 receptor was diminished by chrysin in PCA site. Chrysin reduced histamine release and intracellular calcium level from mast cells. Furthermore, chrysin decreased activation of nuclear factor-kB (NF-kB), and gene expression and secretion of pro-inflammatory cytokine such as, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-4, IL-6 and IL-8, in phorbol 12-myristate 13-acetate and calcium ionophore A23187-stimulated human mast cells. Taken together, chrysin relived allergic inflammation and anti-inflammatory reaction in vivo and in vitro. Our results provide evidence that chrysin could be a beneficial therapeutic drug for mast cell-mediated allergic disorder. Keywords : chrysin; allergic inflammation; histamine; mast cells; inflammatory cytokine.

727 IN VITRO EVALUATION OF LEPTOMYCIN B CYTOTOXIC EFFECTS ON LUNG CANCER CELLS.
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Tumor suppressor p53 can be inactivated through re-localization from the cellular nucleus to the cytoplasm mediated by chromosome maintenance region 1 (CRM1). Leptomycin B (LMB) might serve as a novel class of cancer therapeutics by preventing cytoplasmic localization and inactivation of p53 through the blocking of CRM1. However, the potential of LMB as an anti-cancer agent on lung cancer cell lines has not been investigated. The objective of the present study was to evaluate the cytotoxic effects of LMB on a normal human bronchial epithelial cell line, BEAS-2B, and six human lung cancer cell lines with different p53 status including A549 and H460 (p53 wild type), H226, H522 and H596 (p53 mutant), and H358 (p53 null). Cell lines were treated with 0.1% ethanol (vehicle control), or 0.01-100μM LMB for 4-96 h. Cell cytotoxicity was measured by 3-(4,5-di methylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. The cytotoxic effects of LMB were significantly dose- and time-dependent for both normal and cancer cell lines (P<0.001). However, lung cancer cells exhibited more inhibitory effects as compared to BEAS-2B. For example, the 50% inhibitory concentrations (IC50s) at 48 and 72 h after LMB treat-
and M. E. Andersen. Therefore, we investigated and amplitude but emodepside did not. These observations suggest PF1 may be increased the ryanodine-induced spike amplitude (18.0 ± 3.3 μV, p>0.05, n=5) and frequency (93.0 ± 22.0 to 68.0 ± 24.0 spikes/5min, p<0.05, n=5). PF1 (1 μM) decreased the ryanodine-induced spike amplitude (18.0 ± 3.3 to 12.0 ± 5.9 μV, p<0.01, n=4) and frequency (230.0 ± 68.0 to 36.0 ± 33.0 spikes/5min, p<0.05, n=4). Within 10 min of perfusion, PF1 significantly decreased the spike frequency and amplitude but emodepside did not. These observations suggest PF1 may be more potent or have a different mechanism of action. Therefore, we investigated emodepside effects on voltage-activated potassium currents. Emodepside (1 μM) decreased the voltage-activated potassium currents (1244.0 ± 180.7 to 934.0 ± 160.8 nA, p<0.05, n=6), in contrast to PF1 which increased them. These results demonstrate that emodepside and PF1 do not share the same mode of action. We are currently investigating emodepside effects on voltage-activated calcium currents.

The anti-malarial (AM) drug chloroquine (CQ) reduced steroid levels and disrupted testes development in the male rat fetus at human therapeutic doses. Other AMs, such as quinine, also have been shown to disrupt steroid homeostasis in vitro. The ability of different classes of AM drugs to inhibit testosterone synthesis was tested using an in vitro assay in MA-10 Leydig tumor cells. Three families of AM drugs were evaluated: quinoline based AMs, artemisinin based AMs, and doxycline. Quinoline analogs quinine and its stereoisomer quinidine (Qd), and CQ, a modified quinoline with increased AM efficacy. Artemisinin based drugs have gained favor due to increased efficacy compared to quinoline based drugs, and are particularly effective against multi-drug resistant strains of malaria. Doxycycline (Dox), a broad spectrum antibiotic in the tetracycline family with anti-protozoal activity, is typically used in combination therapy with quinine. Q and Qd inhibited testosterone production with similar potency (EC50 = 3–5 μM); CQ was a stronger inhibitor (EC50 = 1.3 μM). Artemisinin (A), arteunasin (As), and dihydroartemisinin (DHA) were even more potent inhibitors of steroidalogenesis, with EC50 of 0.7, 0.2, and 0.2 μM. In contrast, concentrations up to 100 μM Dox did not reduce testosterone production by the MA-10 cells. The relative potency of the tested AMs for inhibition of steroidalogenesis was: DHA > A > CQ > Qd > Dox. Interestingly, the ability of these AMs to inhibit steroidogenesis in vitro appears to correlate well with their anti-parasitic efficacy. Perhaps, their therapeutic and steroidogenesis-inhibiting effects occur via the same or similar mechanisms.

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POTENT THAN QUININE DERIVATIVES IN INHIBITING TESTOSTERONE PRODUCTION IN MOUSE LEYDIG CELLS.

R. A. Clewell and M. E. Andersen. Computational Biology, The Hanner Institute for Health Sciences, Research Triangle Park, NC.

The anti-malarial (AM) drug chloroquine (CQ) reduced steroid levels and disrupted testes development in the male rat fetus at human therapeutic doses. Other AMs, such as quinine, also have been shown to disrupt steroid homeostasis in vitro. The ability of different classes of AM drugs to inhibit testosterone synthesis was tested using an in vitro assay in MA-10 Leydig tumor cells. Three families of AM drugs were evaluated: quinoline based AMs, artemisinin based AMs, and doxycline. Quinoline analogs quinine and its stereoisomer quinidine (Qd), and CQ, a modified quinoline with increased AM efficacy. Artemisinin based drugs have gained favor due to increased efficacy compared to quinoline based drugs, and are particularly effective against multi-drug resistant strains of malaria. Doxycycline (Dox), a broad spectrum antibiotic in the tetracycline family with anti-protozoal activity, is typically used in combination therapy with quinine. Q and Qd inhibited testosterone production with similar potency (EC50 = 3–5 μM); CQ was a stronger inhibitor (EC50 = 1.3 μM). Artemisinin (A), arteunasin (As), and dihydroartemisinin (DHA) were even more potent inhibitors of steroidalogenesis, with EC50 of 0.7, 0.2, and 0.2 μM. In contrast, concentrations up to 100 μM Dox did not reduce testosterone production by the MA-10 cells. The relative potency of the tested AMs for inhibition of steroidalogenesis was: DHA > A > CQ > Qd > Dox. Interestingly, the ability of these AMs to inhibit steroidogenesis in vitro appears to correlate well with their anti-parasitic efficacy. Perhaps, their therapeutic and steroidogenesis-inhibiting effects occur via the same or similar mechanisms.

728 ARTEMISININ ANTI-MALARIALS ARE MORE POTENT THAN QUININE DERIVATIVES IN INHIBITING TESTOSTERONE PRODUCTION IN MOUSE LEYDIG CELLS.

The anti-malarial (AM) drug chloroquine (CQ) reduced steroid levels and disrupted testes development in the male rat fetus at human therapeutic doses. Other AMs, such as quinine, also have been shown to disrupt steroid homeostasis in vitro. The ability of different classes of AM drugs to inhibit testosterone synthesis was tested using an in vitro assay in MA-10 Leydig tumor cells. Three families of AM drugs were evaluated: quinoline based AMs, artemisinin based AMs, and doxycycline. Quinoline analogs quinine and its stereoisomer quinidine (Qd), and CQ, a modified quinoline with increased AM efficacy. Artemisinin based drugs have gained favor due to increased efficacy compared to quinoline based drugs, and are particularly effective against multi-drug resistant strains of malaria. Doxycycline (Dox), a broad spectrum antibiotic in the tetracycline family with anti-protozoal activity, is typically used in combination therapy with quinine. Q and Qd inhibited testosterone production with similar potency (EC50 = 3–5 μM); CQ was a stronger inhibitor (EC50 = 1.3 μM). Artemisinin (A), arteunasin (As), and dihydroartemisinin (DHA) were even more potent inhibitors of steroidalogenesis, with EC50 of 0.7, 0.2, and 0.2 μM. In contrast, concentrations up to 100 μM Dox did not reduce testosterone production by the MA-10 cells. The relative potency of the tested AMs for inhibition of steroidalogenesis was: DHA > A > CQ > Qd > Dox. Interestingly, the ability of these AMs to inhibit steroidogenesis in vitro appears to correlate well with their anti-parasitic efficacy. Perhaps, their therapeutic and steroidogenesis-inhibiting effects occur via the same or similar mechanisms.

729 EFFECT OF THE CYCLOOXYGENASE-PEPTIDE, EMODEPSIDE, ON VOLTAGE-ACTIVATED CURRENTS IN ASCARIS SUUM.

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Nematode infections are a global problem in human and veterinary medicine. Control is limited by the development of resistance to anthelmintics. Emodepside, an anthelmintic of the cyclooctadepsipeptide group, is effective against benzimidazole-, levamisole- and ivermectin-resistant nematodes in sheep and cattle. Involvement of the calcium-activated potassium channel, SLO-1, in the action of emodepside in Caenorhabditis elegans has recently been reported. Previous studies suggest that the neuropeptide PF1 and emodepside have a similar mode of action. Therefore, we investigated emodepside effects on voltage-activated potassium currents. Emodepside (1 μM) decreased the voltage-activated potassium currents (1244.0 ± 180.7 to 934.0 ± 160.8 nA, p<0.05, n=6), in contrast to PF1 which increased them. These results demonstrate that emodepside and PF1 do not share the same mode of action. We are currently investigating emodepside effects on voltage-activated calcium currents.
cells to secrete chemokines such as interleukin-8 (IL-8) and monocyte chemotactic protein (MCP-1) to recruit inflammatory macrophages and neutrophils to the lung. Inflammatory cells can then release proteases and reactive oxygen species, initiating apoptosis of pulmonary cells, resulting in the destruction of alveolar structure. CS also activates nuclear Nrf2-dependent pathways in pulmonary epithelial cells that increase the expression of cytoprotective enzymes providing a protective mechanism against CS-induced lung inflammation and injury. We hypothesize that activating Nrf2 with sulforaphane (SFN) will afford protection against CS-induced lung damage by increasing Nrf2-dependent gene expression thereby inhibiting chemokine production. Results: Our results indicate that 10 μM SFN does not induce apoptosis in the human epithelial cell line, BEAS-2B cells. SNF triggers Nrf2 translocation to the nucleus after 6 hours as determined by immunoblotting and significantly increases the expression of Nrf2-dependent genes such as NADPH quinone oxidoreductase-1, heme oxygenase-1, and glutamate cysteine ligase modulatory subunit as determined by real-time PCR. BEAS-2B cells exposed to cigarette smoke extract (CSE) lead to a significant increase in IL-8 and MCP-1 levels. Repeated exposures of smokeless tobacco extracts in short term in vitro cultures of human airway epithelial cells to smokeless tobacco products at levels similar to smokeless tobacco extraction demonstrated cytotoxicity and induction of inflammatory gene expression. In conclusion, repeated exposures of smokeless tobacco extracts in short term in vitro cultures of human airway epithelial cells to smokeless tobacco products at levels similar to smokeless tobacco extraction demonstrated cytotoxicity and induction of inflammatory gene expression. However, the mechanism by which the production of chemokines is inhibited through SFN still remains to be elucidated. Moreover, it is unclear whether Nrf2-dependent gene expression induced by SFN is necessary to inhibit chemokines produced by CSE.

### REFERENCES

#### 734 REFERENCE SMOKELESS TOBACCO EXTRACT INDUCED INFLAMMATORY GENE EXPRESSION IN VITRO.

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The use of smokeless tobacco products at the same oral site may result in an acute injury characterized by ulceration and inflammation that is reversible upon elimination of the same site use. However, little research has been conducted on in vitro models that mimic the human oral cavity response to smokeless tobacco exposure. In the current study, we used the HET-1A cell line to investigate the role of short term repeated exposures to reference smokeless tobacco extracts similar to product usage in humans. The total exposure lasted for 3 to 5 hours and consisted of 1hr of exposure followed by 1 hr of recovery followed by 1 hr of exposure (3hrs) followed by 1 hr of recovery followed by 1 hr of exposure (5hrs). Results from this exposure method were compared to those from the cells exposed for the entire 3 to 5hrs. The use of the repeated dosing in vitro model resulted in reduced cytotoxicity as detected by Calcin AM staining, yet augmented the inflammatory gene expression of IL-6, IL-8, Cox-2, and TNF-alpha detected by RT-PCR. To investigate the smokeless tobacco components that may be involved in the cytotoxicity and inflammatory gene expression, nicotine and hyperosmolarity were investigated using this model. Our findings indicate that exposure to nicotine alone, at the levels in smokeless tobacco extracts used in these studies, did not cause cytotoxicity or inflammatory gene induction in this in vitro model. However, HET-1A cells exposed to hyperosmotic solutions at levels similar to smokeless tobacco extracts demonstrated cytotoxicity and induction of inflammatory gene expression. In conclusion, repeated exposures of smokeless tobacco extracts in short term in vitro cultures of HET-1A enhance inflammatory gene expression and hyperosmolarity appears to play a role in this process.

### 735 IL-17 MEDIATED INFLAMMATORY RESPONSE INDUCES POLYMERIC Ig RECEPTOR AND ELEVATED IGA LEVELS IN SILICA EXPOSED Rag1/-/- MICE.

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Prolonged exposure to crystalline silica (SiO2) in occupational and environmental settings induces an inflammatory lung disease (known as silicosis) characterized by a diffuse mononuclear cell infiltrate in the lung that can progress to pulmonary fibrosis with chronic exposure. Insufficient information on the pathophysiological mechanisms of silicosis has severely limited the development of effective therapeutic strategies. Because conventional anti-inflammatory agents do not control SiO2-induced inflammation, much less cure the disease, more effective therapeutic alternatives must be developed. IL-17 plays a prominent role in the pathogenesis of lung inflammatory diseases such as asthma, chronic obstructive pulmonary disease, and cystic fibrosis, by promoting the recruitment and survival of neutrophils, as well as the establishment of chronic inflammation. However, it remains unclear whether IL-17 contributes to SiO2-induced neutrophilia and chronic inflammation. In the current study, we investigated the inflamed airways of SiO2 exposed lymphopenic Rag1/-/- and C57Bl/6 wild-type mice. SiO2 exposure results in elevated neutrophil-dominated inflammation associated with elevated levels of IL-17 in the lavage. Coincident with this inflammatory response, was a striking induction of inflammatory cytokines such as IL-17 in the lung. Inflammatory cells can then release proteases and reactive oxygen species, initiating apoptosis of pulmonary cells, resulting in the destruction of alveolar structure. CS also activates nuclear Nrf2-dependent pathways in pulmonary epithelial cells. Our results indicate that 10 μM SFN does not induce apoptosis in the human epithelial cell line, BEAS-2B cells. SNF triggers Nrf2 translocation to the nucleus after 6 hours as determined by immunoblotting and significantly increases the expression of Nrf2-dependent genes such as NADPH quinone oxidoreductase-1, heme oxygenase-1, and glutamate cysteine ligase modulatory subunit as determined by real-time PCR. BEAS-2B cells exposed to cigarette smoke extract (CSE) lead to a significant increase in IL-8 and MCP-1 levels. Repeated exposures of smokeless tobacco extracts in short term in vitro cultures of human airway epithelial cells to smokeless tobacco products at levels similar to smokeless tobacco extraction demonstrated cytotoxicity and induction of inflammatory gene expression. In conclusion, repeated exposures of smokeless tobacco extracts in short term in vitro cultures of HET-1A enhance inflammatory gene expression and hyperosmolarity appears to play a role in this process.

### 736 DIFFERENTIAL EFFECTS OF 1-NITROPYRENE AND 1-AMINOPYRENE ON CXCL8 (IL-8) AND CCL5 (RANTES) IN BEAS-2B CELLS: ROLE OF AHR, ARNT, NFκB AND AP-1.

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1-Nitropyrene (1-NP) is an abundant PAH in diesel exhaust particles (DEPs) and has been reported to be among the main contributors to the mutagenicity of DEPs. The nitro group on 1-NP can be reduced by cytoxic nitroreductases, giving rise to 1-aminopyrene (1-AP) through a process that may cause formation of reactive oxygen species. We have recently shown that 1-NP may be a potent inducer of cytokine and chemokine responses in human bronchial epithelial BEAS-2B cells. In the present study, we investigated the effects of 1-NP and 1-AP on 17 cytokine and chemokine genes in BEAS-2B cells by real-time PCR. While the 1-NP-induced response was characterized by maximum effects on CXCL8 (IL-8) and TNF-α expression, 1-AP induced a completely different gene expression pattern dominated by CCL5 (RANTES) and CXCL10 (IP-10). This marked difference in response pattern following 1-NP and 1-AP exposure was confirmed by ELISA on CXCL8 and CCL5. Real-time-PCR further showed that the two compounds did not induce expression of aryl hydrocarbon receptor (AhR)-regulated genes, such as CYP1A1 and CYP1B1. In spite of this, silencing of AhR and the AhR nuclear transporter (ARNT) by siRNA increased the release of CCL5 and CXCL8, respectively. Preliminary findings by “transcription factor ELISA” suggest that 1-NP induced a stronger activation of activator protein-1 (AP-1) than 1-AP, while 1-AP induced the...
strongest activation of nuclear factor-κB (NF-κB). In conclusion, we show that 1-β-glucan induces different cytokine/chemokine expression patterns in BEAS-2B cells. This seems to be linked to differential activation of transcription factors including NF-κB and AP-1. Furthermore we hypothesize that the effects of silencing AhR and ARNT on chemokine release are due to interactions between AhR and NF-κB.

738 ROLE OF SURFACTANT PROTEIN D IN OZONE-INDUCED LUNG INJURY AND INFLAMMATION.

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Ozone is a ubiquitous urban air pollutant known to induce lung injury and inflammation. This is associated with the release of nitrogen species (RNS) which contribute to toxicity. Surfactant protein-D (SP-D) functions to down regulate inflammation. We examined the role of SP-D in ozone-induced lung toxicity. Exposure of mice to ozone (0.8 ppm, 3h) resulted in RNS-mediated posttranslational modifications of SP-D causing it to become a pro-inflammatory mediator, a possible mechanism whereby RNS induce pulmonary inflammation. To investigate this, we used SP-D−/− mice. Ozone inhalation resulted in increased BAL protein levels and macrophage content, markers of lung inflammation and injury in both wild type and SP-D−/− mice, 72 hr post exposure. These effects were more pronounced in SP-D−/− mice. Greater levels of NOx were also detected in BAL from SP-D−/− mice, as well as increased numbers of iNOS positive macrophages in lung tissue. Using a SCIREQ FlexiVent, we next analysed pulmonary function. Total lung resistance (RL) increased in both WT and SP-D−/− mice in response to increasing positive end expiratory pressure (PEEP). This was due to increases in central airway resistance (Rn) and static compliance (Cst). Whereas RL was greater in SP-D−/− mice when compared to WT mice, at all PEEPs, Cst values were only greater at low PEEPs. These results are consistent with an emphysematous morphology in lungs of SP-D−/− mice. Exposure of WT mice to ozone resulted in increased RL with little change in Cst. At low PEEPs, Rn also increased in WT mice following ozone inhalation. These findings indicate that ozone induces restrictive lung disease. Exposure of SP-D−/− mice to ozone resulted in decreases in RL and Cst to levels observed in ozone exposed WT mice, indicating that the effects of ozone on respiratory mechanics are more significant than the loss of SP-D. These results suggest that SP-D plays distinct roles in ozone-induced pulmonary inflammation and altered lung function.

739 IDENTIFICATION OF CYTOKINES AND GROWTH FACTORS ASSOCIATED WITH EXPOSURE OF MESOTHELIAL CELLS TO ASBESTOS AND PROGRESSION OF MESOTHELIOMA GROWTH IN MICE.

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The mechanisms by which pathogenic particulates such as asbestos cause injury to lung and pleural cells remain unclear, although chronic inflammation has been linked to initiation and progression of numerous cancers, including malignant mesothelioma (MM). The purpose of the studies described here was to examine the ability of a contact-immortalized human peritoneal mesothelial cell line (LP9/TERT-1) and two human MM cell lines (PPM Mill, Hmeso) to release cytokines/growth factors in response to either crocidolite asbestos exposure in vitro, or inoculation of MM cells into immunodeficient SCID mice. In vitro Bio-Plex studies demonstrated that exposure of LP9/TERT-1 cells to asbestos increased the amount of IL-1β, IL-6, IL-13, bFGF, G-CSF, and VEGF secreted into medium, suggesting that asbestos may elicit a number of autocrine growth factor pathways in mesothelial cells. Peritoneal xenograft studies in SCID mice using PPM Mill and Hmeso cells revealed enhanced cell injury as illustrated by increases in peritoneal lavage fluid (PLF) lactate dehydrogenase levels, neutrophilia as determined by cell differential counts of PLF cytopsins, and increases in IL-1β, IL-6, IL-7, IL-8, IL-12 (p70), MCAF, and VEGF at 4 weeks post-inoculation as shown via Bio-Plex analysis of PLF. These changes corresponded with the time-dependent establishment of tumor sphericals and mesenteric masses within the peritoneal cavity. Overall, these results demonstrate that human mesothelial and MM cells have independent roles in producing cytokines/growth factors linked to inflammation and carcinogenesis. Additionally, determining early molecular responses to crocidolite asbestos exposure and MM tumor formation may directly contribute to understanding the etiology of this disease and aid in identifying targets for therapeutic intervention.


740 INHIBITION OF CF AIRWAY HMGB1 REDUCES P. AERUGINOSA INFECTION AND NEUTROPHILIC INFLAMMATORY LUNG INJURY.

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Despite advances in the understanding of the pathogenesis of CF and the improvement in its management, the average life span of individuals with CF remains short. Chronic pulmonary infection with Pseudomonas (P.) aeruginosa and persistent neutrophilic lung inflammation contribute to the morbidity and mortality associated with CF patients. High mobility box gene 1 (HMGB1), a proinflammatory cytokine, has recently been implicated in mediating neutrophilic inflammation in CF in the absence of infection. We show here that HMGB1 levels were elevated in bronchoalveolar lavage fluids (BAL) of CF patients and CFTR−/− mice. In both WT and CF mice infected with P. aeruginosa, treatment with neutralizing monoclonal anti-HMGB1 antibody significantly reduced neutrophilic infiltration, bacterial burden and injury in the lung. Notably, recombiant HMGB1 directly inhibited the ability of isolated macrophages to phagocytose and kill P. aeruginosa. A similar suppression in macrophages’ ability to phagocytose bacteria was invoked by BAL from CF patients and this suppression was reversed by HMGB1-neutralizing antibodies. Interestingly, toll-like receptor 4 (TLR4) on macrophages plays an essential role in signaling HMGB1-mediated macrophage dysfunction. These findings suggest that HMGB1 and downstream signaling molecule TLR4 could provide novel therapeutic targets for reducing both lung inflammation and bacterial infection in CF patients.

741 RODENT INHALATION STUDIES AND CIGARETTE SMOKE INFLAMMATION-MEDIATED PROMOTION OF TUMOR CELLS.


A rodent inhalation model capable of reproducibly demonstrating dose-dependent increases for lung tumor development in response to cigarette smoke exposure has yet to be developed. Model development efforts have generally presumed that cigarette smoke mediates lung tumor development primarily through genetic damage.
However, review of published and emerging rodent inhalation data, in terms of mode-of-action, suggests that cigarette smoke exerts it toxicological effects primarily through inflammation-mediated promotion of tumor cell survival and/or clonal expansion. The conclusion is similarly supported by epidemiological data analyzed according to the multi-stage carcinogenesis model, as well as emerging evidence regarding the roles for inflammation and immunodetecting during tumor promotion. Accordingly, efforts were undertaken to develop a promotion-initiation (inhalation) model that demonstrates dose-dependent increases for lung tumor development. Initial studies evaluated mouse strains reportedly sensitive to lung tumor development, multiple tumor initiators, and a cigarette smoke exposure regimen previously shown to induce both lung cell proliferation and apoptosis. Subsequent efforts entailed refinement of the testing protocols for mouse strains demonstrating cigarette smoke-mediated effects. RasH2 mice exhibited dose-dependent and statistically significant increases for lung tumor development using 7,12-dimethylbenz[a]anthracene and cigarette smoke as tumor initiator and promotor, respectively. Tumor multiplicities for sham- and cigarette smoke-exposed (0.16, 0.28 and 0.40 mg WTPM/L) mice were 0.92±0.24, 0.64±0.24, 1.42±0.26 and 1.86±0.33 (means+/-s.d.), respectively. The range of responses substantially increased (i.e., from 2- to 5-fold) by varying the initiator concentration and retaining a constant cigarette smoke concentration; this approach provided dose-dependent and statistically significant findings.

**744 CLASSICAL AND ALTERNATIVE ACTIVATION OF RAT LIVER KUPFPER CELLS AND ENDOTHELIAL CELLS: IMPACT OF ACETAMINOPHEN (APAP).**

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Macrophages play a key role in regulating the immune system, ensuring a balance between pro- and anti-inflammatory reactions. Evidence suggests that this is mediated by distinct subsets which are broadly classified as classically (M1) or alternatively (M2) activated. Whereas M1 macrophages display a cytotoxic/proinflammatory phenotype, M2 macrophages suppress inflammation and initiate wound repair. The role of these cells in APAP induced hepatotoxicity is unknown. We speculate that liver macrophages differentiate into these two phenotypes following APAP overdose and it is the relative activity of these cells that determines the outcome of the toxic response. To test this, we exposed rat Kupffer cells to M1 (IFNγ and LPS) or M2 (IL-10 or IL-4/IL-13) inducing stimuli which are known to be released during hepatic inflammatory responses to APAP. M1 phenotype was assessed by expression of inducible nitric oxide synthase (iNOS), and M2 phenotype by expression of arginase-1 (Arg1) and mannose receptor (MR). IFNγ (20 ng/ml) and LPS (100 ng/ml) markedly induced iNOS expression in Kupffer cells. This was associated with suppression of the M2 marker, MR. These effects were most pronounced after 48 hr in culture. In contrast, IL-10 (10 ng/ml) and IL-4/IL-13 (10 ng/ml) upregulated Arg1 and MR expression in liver macrophages, indicating induction of an M2 phenotype. Surprisingly, liver endothelial cells exhibited similar responses to these mediators demonstrating that these cells are also capable of classical and alternative activation. We also found that co-culture of Kupffer cells with hepatocytes treated with 5 mM APAP for 2 hr enhanced IL-10 or IL-4/IL-13 induced Arg1 expression. These results demonstrate that both liver macrophages and endothelial cells can be classically and alternatively activated by inflammatory mediators. Moreover, APAP stressed hepatocytes facilitate the induction of M2 macrophage phenotype. Supported by GM034310, ES004738, CA132624, AR055073 and ES005022.

**745 MAPK1 IS REQUIRED FOR MACROPHAGE ACTIVATION BY FACTORS RELEASED FROM ACETAMINOPHEN-INJURED HEPATOCYTES. POTENTIAL ROLE OF HMBG1.**

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Toxic doses of the analgesic acetaminophen (AA) are known to cause centrilobular hepatic necrosis. Accumulating evidence indicates that activated macrophages and inflammatory mediators they release are involved in the pathogenic process. Mitogen-activated protein kinase-1 (MAPK1) plays an important role in macrophage activation during inflammation. HMGB1 is a nuclear protein that is passively released following necrotic cell death and can activate macrophages. We have previously found that hepatocytes treated with AA release HMGB1 which upregulates macrophage expression of inflammatory mediators. In the present studies, we investigated mechanisms mediating these effects. Macrophages were incubated with conditioned medium collected from hepatocytes (HCM) treated with control (C) or AA (5 mM) for 24 hr. HCM-AA, but not HCM-C, was found to induce expression of phosphorylated MAPK1 in macrophages. This was evident within 30 min and persisted up to 6 hr and was associated with increased mRNA expression for the HMGB1 receptors, RAGE and TLR4. HCM-AA also upregulated expression of the proinflammatory cytokines, IL-6, MIP-1α and MIP-2, as well as matrix metalloproteinase-13 (MMP-13) and cyclooxygenase-2 (COX-2), a key enzyme in prostaglandin biosynthesis. Pretreatment of macrophages with the MAPK1 inhibitor U0126 abrogated the effects of HCM-AA on the expression of RAGE, COX-2, MIP-1α, MIP-2 and MMP13. Together, these results suggest that MAPK1 is important for HMGB1-dependent macrophage activation during AA-induced hepatotoxicity. Supported by NIH GM034310, ES004738, CA132624, AR055073 and ES005022.

**743 POLYCHLORINATED BIPHENOLS (PCB) INDUCE TUMOR NECROSIS FACTOR α (TNFα) PRODUCTION IN RAW 264.7 CELLS.**

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Epidemiological studies have identified multiple PCBs which are associated with increased risk for liver injury, insulin resistance and the metabolic syndrome. Because pro-inflammatory stimuli are central to the pathogenesis of these diseases and monocyte priming by toxics such as ethanol are shown to increase lipopolysaccharide (LPS)-stimulated TNFα production, we tested the hypothesis that treatment with PCBs could stimulate basal and LPS-induced TNFα production from monocytes. 15 PCBs were selected which epidemiological studies have shown are associated with elevated serum liver enzymes. RAW 264.7 cells were treated with either PCB, LPS or a combination of both compounds, and the TNFα levels were determined in the cell culture medium. At these concentrations, the PCBs were not cytotoxic as determined by MTT assay. Treatment with PCBs increased TNFα production either basally or under the condition of co-treatment with LPS. Increases in TNFα production were paralleled by increases in MCP-1 and MIP-2. Both coplanar and non-coplanar PCBs increased TNFα production. The results indicate that PCBs treatment can increase pro-inflammatory cytokine production from RAW 264.7 cells and may be able to prime these cells in a “two hit” disease model.
MACROPHAGE INHIBITORY CYTOKINE-1 (MIC-1) AND SUBSEQUENT PRODUCTS MEDIATE EPITHELIAL TUMOR CELL DEATH RESPONSES BY THERAPEUTIC RIBOTOXIC INSULT.

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Mucosal ribotoxic stresses pose a potent suppressive activity against epithelial tumor cell progression. In the present study, we demonstrated that macrophage inhibitory cytokine 1 (MIC-1) and its associated signals determined the colon cancer cell response to the therapeutic ribotoxic. The ribotoxic stress agent anisomycin induced MIC-1 gene expression which was involved in the therapeutic-triggered cell death pathway. MIC-1 was also a critical inducer of apoptosis-related gene products such as activated urokinase-type plasminogen activator (PLAU) and PLAU receptor (uPAR). When MIC-1 or PLAU action was repressed in the epithelial tumor cells, anisomycin triggered a survival-related MAP kinase such as ERK. Mechanically, gene expression of apoptosis-mediator MIC-1 was enhanced by Activating transcription factor 3 (ATF-3) via the p38 MAP kinase signaling pathway. In conclusion, ribotoxic anisomycin induced MIC-1 expression via p38-ATF3 pathway and subsequent apoptosis while suppressing survival ERK signal in the colon cancer cells. The results of this study provide mechanistic insight into epithelial tumor cell decision for death or survival pathways in response to ribotoxic chemo-therapeutics. This work was supported by the Korea Research Foundation (KRF) grant funded by the Korea government (MEST) (No.2009-0087028)

NUCLEAR FACTOR ERYTHROID 2 (NF-E2): IDENTIFICATION OF A NOVEL IMMUNOMODULATOR THAT REGULATES PULMONARY INFLAMMATION.

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Delayed polymorphonuclear leukocytes (PMNs) apoptosis during immune complex-mediated lung injury contributes to lung damage. Induction of PMN apoptosis is an efficient mechanism of resolving inflammation and limiting tissue injury. Nuclear Factor Erythroid 2 (NF-E2) was identified as an apoptosis regulating protein in neutrophils. We demonstrated its presence in cytosol and in secretory vesicles and azurophilic granules of unstimulated PMNs. Upon fMLP stimulation, NF-E2 translocated to the nucleus and induced NF-E2 DNA-binding activity. This study provides mechanistic insights into the role of NF-E2 in apoptosis prevention of colon cancer. Thus, we hypothesized that phosphorylated NF-E2 but not pRSET vector control, to stimulate actin polymerization and human neutrophil chemotaxis. Furthermore, in vitro overnight incubation of rNF-E2 but not pRSET vector control, with human PMNs promoted neutrophil survival to the same extent as LPS treatment. Thus, collectively, these data suggest secreted NF-E2 promotes neutrophil survival by acting as an alarm during inflammation.

ANTI-INFLAMMATORY AND ANTI-CANCER EFFECTS OF POLYPHENOLICS FROM YAUPON HOLLY (ILEX VOMITORIA) IN COLON CELLS.

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Chronic intestinal inflammation is a significant risk factor for colon cancer and polyphenolics in general have been demonstrated to have anti-inflammatory properties. Our aim was to assess the chemopreventive potential of polyphenolics extracted from Yaupon Holly leaf (Yp) (Ilex vomitoria) and compare the anti-inflammatory and anti-cancer properties to green tea (Camellia sinensis) (GT) in HT-29 colon cancer and normal CCD-18Co cells. Polyphenolic fractions from GT and Yp were analyzed by HPLC and LC/ESI/MSn effect on cell viability was assessed by MTT. Fractions based on the MTT assay, one Yp fraction was selected to investigate its anti-inflammatory and immunomodulatory effects on CCD-18Co cells. The inhibition of induced reactive oxygen species (ROS) was assessed with the dichlorofluorescein assay. The induction of glutathion S-transferase (GST) and glutathion peroxidase (GPx) activities were assessed spectrophotometrically, and modulation of protein and gene expression and microRNA by western blots and qRT-PCR respectively. Yp protected normal colon CCD-18Co cells against ROS compared to HT-29 cells and increased the activity of both GPX and GST by 1.8 fold. The protection exerted by these compounds on CCD-18Co cells was linked to inhibition of AhR gene expression up to 0.2 fold of untreated cells, which was linked to the decreased expression of Phase I enzymes CYP1A1 and CYP1B1 up to 0.35 fold of control. Furthermore, Yp decreased LPS-induced NF-kB gene expression and NF-kBp65 activation and the downstream pro-inflammatory COX-2 expression and prostaglandins (PGE2) protein up to basal levels of LPS-un-treated cells. The LPS-exerted Toll Like Receptor-4 (TLR4) was significantly inhibited at gene expression level (by 0.6 fold) and protein. MicroRNA146a, which has a target sequence in the mRNA of TLR-4, was induced up to 3 fold, potentially as part of a negative feedback loop regulation of LPS-induced NF-kB activation. Overall, these results may be of clinical relevance in the mitigation of inflammatory bowel conditions and prevention of colon cancer.

CHARACTERIZATION OF AN INFLAMMATORY STRESS MODEL OF AMIODARONE IDIOSYNCRATIC HEPATOTOXICITY IN RATS.

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Amiodarone (AMD), a class III antiarrhythmic drug, causes idiosyncratic hepatotoxicity in human patients. We demonstrated the pathophysiological stress caused by lipopolysaccharide (LPS) renders otherwise nontoxic doses of AMD hepatotoxic in rats. We characterized this model further and tested the hypothesis that LPS-induced changes in tumor necrosis factor-alpha (TNF) and coagulation system activation are affected by AMD. Male, SD rats were treated with nonhepatotoxic doses of AMD or its vehicle IP and with LPS or saline IV. Elevated serum alanine aminotransferase (ALT) activity and midzonal hepaticcellular necrosis in H&E-stained liver sections were observed only in AMD/LPS-cotreated rats. The time of AMD administration relative to LPS was critical to the development of liver injury: AMD injected 16h before LPS increased ALT activity, whereas AMD
injected 2h to 12h before LPS failed to cause this response. The increase in ALT activity in AMD/LPS-cotreated rats depended on the dose of AMD as well as LPS. A treatment system. In summary, AMD treatment during modest inflammation can induce increased by LPS and was slightly prolonged by AMD. In a murine hepatitis cell line in vitro, AMD caused dose-dependent cytotoxicity that was increased by addition of TNF. In rats, thrombin-antithrombin complex in the plasma was significantly elevated in the AMD/LPS cotreatment group, indicating activation of the coagulation system. In summary, AMD treatment during modest inflammation can induce severe hepatotoxicity in rats, and TNF and coagulation system activation are associated with the induction of liver injury in this animal model of human idiopathic AMD-induced liver injury. (Supported by NIH R21GM078065)

751 INTEGRATION OF MICROARRAY AND PROTEOMIC DATA REVEALS SUSCEPTIBILITY FACTORS FOR EXACERBATED IMMUNE RESPONSE IN DIET-INDUCED OBESE MICE EXPOSED TO CIGARETTE SMOKE.
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Pacific Northwest National Laboratory, Richland, WA and Battelle Toxicology Northwest, Richland, WA.

Smoking and obesity are two of the most important, preventable risk factors for human morbidity and mortality. Chronic inflammation and oxidative stress appear to be unifying mechanisms underlying the interaction of these life-style-induced risk factors with the genome, resulting in a variety of chronic human diseases. To identify the key biological pathways that define environmental and lifestyle susceptibility factors, we performed parallel exposures of normal weight (NW) and diet-induced obese (DIO) C57BL/6 mice to (250μg/L WTPM) mainstream or (85μg/L WTPM) sidestream cigarette smoke for 6h/6d for 8d. Bronchoalveolar lavage (BAL) cytology indicated a strong neutrophilic response to smoke exposure, which was 3.5-fold higher in DIO mice than NW. Microarray analysis revealed that DIO reprograms the lung's transcriptional response, altering both the number of genes and the specific molecular pathways induced or suppressed by smoke exposure. Global proteomics analysis of lung tissue identified almost 5000 proteins, some of which are known to be secreted into the BAL fluid. Pathway analysis revealed an overall suppression of the immune system in DIO sham control animals compared to NW controls, which was accompanied by an increase in basal expression of several heat shock proteins. Statistical integration of these data identified several gene and protein markers that are predictive of the susceptibility phenotype, providing biosignatures of systemic chronic inflammation and oxidative stress. These results show enhanced and unique stress responses in obese mice, which make them sensitive to environmental exposure of lung toxicants. Supported by U54 ES016015.

752 IDENTIFICATION OF MODIFIED LUNG PROTEINS AS BIOMARKERS OF SYSTEMIC CHRONIC INFLAMMATORY AND OXIDATIVE STRESS IN MICE.
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Pacific Northwest National Laboratory, Richland, WA and Battelle Toxicology Northwest, Richland, WA.

Smoking and obesity are two of the most important, preventable risk factors for human morbidity and mortality. Chronic inflammation and oxidative stress appear to be the mechanisms underlying these risk factors resulting in a variety of chronic human diseases. To identify oxidative protein modifications a C57BL/6 mice to mainstream (MS) (250μg/L WTPM) and sidestream (SS) (85μg/L WTPM) cigarette smoke were exposed resulting in >750 unique modified peptides identified for DOPA, Dopamine (DQ), and nitro, amino, bromo, and chloro tyrosine modifications. Multiple proteins of interest were seen with a diversity of modifications. For example, cytoskeletal regulatory 14-3-3 protein zeta/delta contained both DQ and nitrotyrosine. Cytochrome C oxidase and transglutamin-3 were also detected with DQ and nitrotyrosine. Heat-shock protein beta-1 and retinoic acid-induced protein 3 were also found with both DQ/nitrotyrosine and DQ/bromotyrosine modifications respectively. Statistical analyses of individual biological samples are currently being investigated. The identified oxidative modifications provide insights into systemic chronic inflammatory responses while many of the proteins may constitute potential biomarkers of environmental stress. Supported by ES016015.

753 CYTOTOXIC RESPONSES TO REFERENCE MOIST SMOKELESS TOBACCO EXTRACTS IN A THREE DIMENSIONAL ORAL CELL CULTURE SYSTEM (EPIORAL\textsuperscript{TM}).
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The use of smokeless tobacco products at the same oral site may result in an acute injury characterized by ulceration and inflammation that is reversible upon elimination of same site use. However, little research has been conducted on in vitro models that mimic the human oral cavity response to smokeless tobacco exposure. In the current study we used the 3D oral mucosal system, EpiOralTM, to investigate the responses of oral mucosal tissue to highly concentrated extracts of reference moist smokeless tobacco (MST) that reflect exposure at the site of MST use in the human oral cavity. The responses observed following 16h of MST exposure included loss of cell viability (detected by MTT), an increase in the small inter- nal heat shock protein gene expression (detected by RT-PCR) and protein release (detected by cytometric bead assay and ELISA) and ultrastructural changes as measured by scanning electron and light microscopy. In addition, 2D differential gel electrophoresis was conducted on the culture supernatant and identified 31 unique proteins that were differentially modulated by MST extracts. The released proteins were analyzed for their associated functional class and revealed proteins involved in proteolysis, oxidative stress, calcium binding and cytoskeletal function. In conclusion, responses observed in the EpiOralTM model system are similar to those reported for the human oral mucosa during MST use. Thus, the EpiOralTM system is a promising model for evaluating tissue responses to smokeless tobacco extracts.

754 ROLE OF CRYPT PANETH CELLS IN INTESTINAL INFLAMMATORY RESPONSE TO TOTAL-BODY GAMMA-IRRADIATION.
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Ionizing irradiation (IR) can ablate bone marrow, produce immunotoxic effects, and damage the intestinal crypt/villus system. The subsequent inflammation is often associated with a compromise of the intestinal mucosal barrier function. The objective of this work is to investigate the role of innate defense mechanisms mediated by the host cells (a process that is assessed by RT-PCR) in the development of a host cell response to inflammatory (IL-β) and oxidative stress, calcium binding and cytoskeletal function. In conclusion, responses observed in the EpiOralTM model system are similar to those reported for the human oral mucosa during MST use. Thus, the EpiOralTM system is a promising model for evaluating tissue responses to smokeless tobacco extracts.

755 AN AUTOMATED, QUANTITATIVE HIGH-CONTENT CELL-BASED IMAGING ASSAY FOR CHEMOTAXIS.
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Inhibition of cell migration holds great promise for treating inflammatory disorders as well as other pathological conditions where inflammation is a contributing factor. Developing such therapies requires robust and scalable methods for functional

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AHR ACTIVATION SUPPRESSES COLITIS BY INHIBITING INFLAMMATORY TH1 CELLS THROUGH INDUCTION OF FOXP3+ REGULATORY T CELLS AND TH17 CELLS.

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Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn’s disease (CD), are associated with chronic inflammation of the intestinal tract. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a potent environmental contaminant that binds to AhR with high affinity. Recent studies have indicated that AhR activation may regulate some important components of the immune system. In the current study, we investigated the effect of AhR activation on dextran sodium sulphate (DSS)-induced colitis in mice. BL/6 mice were administered with 3% DSS in water by ad libitum followed by treatment with TCDD (25 microgram/kg body weight). AhR activation effectively attenuated the overall clinical score as well as various pathological parameters of colitis. Also, it reversed the colitis-associated decrease in body weight and increase in serum (IL-6, MCP-1, KC, Eotaxin and TNF-alpha) markers of colitis. Also, it reversed the colitis-associated decrease in body weight and increase in serum (IL-6, MCP-1, KC, Eotaxin and TNF-alpha) markers of colitis. This study suggests that AhR activation may serve as a novel therapeutic target for the chronic inflammatory bowel disease (Supported in part by NIH grants R01ES09908, R01AI058300, R01DA016545, and P01AT003961).

ENDOTOXINS IN AFRICAN DUST (PM10): POSSIBLE IMPLICATION IN PUERTO RICAN ASTHMA EXACERBATION.

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Asthma is a chronic inflammatory disease of the airways whose prevalence in the US has considerably increased in minority groups, particularly in Puerto Rican children. African (Saharan) dust, impacts areas (Caribbean) reported with some of the highest worldwide incidences of asthma. A popular belief in PR is that Saharan Dust Events (SDE) are associated with increased allergic and asthma attacks, however, no systematic study has yet been able to connect them. Endotoxins (ENX) in airborne particulate matter (PM10) are among the environmental factors associated with increased asthma. This study evaluates the cytotoxic and pro-inflammatory role of ENX present in PM10 of Saharan dust and its relationship to pediatric asthma exacerbation in PR. To accomplish this, we examined PM10 from the Atlantic Ocean and 2 inland sites (Fajardo/rural and Guaynabo/urban) during 2004. PM10 and ENX levels increased when SDE reached the shores of Puerto Rico and ENX was found to be higher in PM10 Guaynabo aqueous extracts. A retrospective analysis of pediatric asthma claims, using ASE (Health Insurance Administration of PR) database revealed that March 2004 contained the highest number of visits at both sites. This also correlates with one of the highest number of SDE reaching PR. An in-vitro model of lung epithelial cells (BEAS-2B) was used to evaluate cytotoxic and pro-inflammatory response (IL-6 and IL-8 release) of SDE
The molecular mechanisms by which PM2.5 promotes inflammatory responses are not well understood. Recent data suggests that the mechanisms controlling mRNA stability are crucially involved in determining the levels of cytokines gene expression during an acute inflammatory response. However, there is no experimental evidence on the effects of PM2.5 in the mRNA half-lives of cytokines. Therefore, the main goal of this research was to determine the effects of PM2.5 in the mRNA stabilization of pro-inflammatory cytokines. Time course experiments were conducted in a human bronchial epithelial cell line exposed to diesel exhaust particles, a standard reference material of urban dust, and to organic extracts of ambient PM2.5 collected in Puerto Rico. Induction of IL-6 and IL-8 mRNA levels were observed as early as 30 min of exposure. Preliminary results demonstrated increments in the half-lives of IL-6 and IL-8 mRNAs of cells exposed with PM2.5. These data suggest that mRNA stabilization of cytokines is one of the molecular mechanisms by which PM2.5 induces acute inflammatory responses in the lung.

**761 DEVELOPMENT OF NOVEL LC-MS/MS METHOD TO QUANTIFY F2-ISOPROSTANES IN BIOLOGICAL SAMPLES.**


Oxidative stress has been implicated in a number of incidents of drug-induced liver injury. One way to quantify oxidative injury is to measure endogenous products derived from lipid peroxidation, such as F2-isoprostanes (F2-IsopPs), a group of prostaglandin F2-like compounds formed by non-enzymatic oxidation of arachidononic acid. The goal of this study was to establish a novel analytical method to determine the stability of F2-IsopPs in biological samples. LC-MS/MS-based method was developed because of its excellent accuracy, reproducibility and is adaptable to high throughput. Samples (either plasma, urine or tissue homogenate) were first subjected to protein precipitation, followed by solid phase extraction. The chromatographic eluent was directly injected to an Agilent 6410 Triple-Q mass spectrometer that was operated using an electrospray ionization source in the negative ion mode (ESI-) for multiple reactions monitoring (MRM). Systematic method optimization was performed to achieve the complete separation of the six targeted F2-IsopPs by HPLC and the highest sensitivity for each F2-IsopPs by mass spectrometry quantification. The linearity of the method was proved for all six F2-IsopPs over the full calibration range (1 to 1000 ng/mL) with r2>0.9. The method is currently being used to quantify the six F2-IsopPs in plasma, urine and liver samples from rats treated with valproic acid.

**762 NADPH OXIDASES ARE CRITICAL TARGETS FOR PREVENTION OF ETHANOL-INDUCED BONE LOSS.**

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The molecular mechanisms through which chronic alcohol consumption induce bone loss and osteoporosis are largely unknown. Ethanol increases expression and activates NADPH (nicotinamide adenine dinucleotide phosphate) oxidase enzymes (Nox) in osteoblasts leading to accumulation of reactive oxygen species. This might be the initiating step in inhibition of bone formation and stimulation of bone resorption. Using cycling female Sprague-Dawley rats treated with ethanol (12 g/kg/d) using total enteral nutrition, we found that EtOH treatment for 28 d reduced trabecular bone mineral density (BMD) (P<0.05). EtOH effects on BMD were blocked by exogenous 17-β estradiol (25 mg/kg/d, s.c.) (P<0.05). Moreover, co-administration of the antioxidant N-acetylcysteine (NAC, 2 g/kg/d), or diphenylene iodonium (DPI, 1 mg/kg/d, s.c.) a pan-Nox inhibitor also abolished chronic EtOH-induced bone loss. EtOH treatment was associated with up-regulation of mRNA levels in bone of three Nox subtypes 1, 2, 4 and RANKL (receptor activator of NF-κB ligand), an essential factor for differentiation of bone marrow monocyte-macrophage lineage cells into osteoclasts (P<0.05). Protein expression of Nox 4, a constitutively active Nox isoform expressed in non-phagocytic cells, was also up-regulated by EtOH in bone (P<0.05). All three compounds, 17-β estradiol, NAC and DPI were able to block EtOH-induced up-regulation of Nox and RANKL. In vitro studies using pre-osteoblastic ST2 stromal cells and osteoblastic UMR-106 cells demonstrated that 50 mM EtOH directly up-regulated Nox expression in osteoblasts (P<0.05). Furthermore, DPI dose-dependently blocked EtOH and hydrogen peroxide-induced RANKL gene expression and activated RANKL promoter activity in osteoblasts (P<0.05). These data demonstrate a critical role for Nox in EtOH-induced osteoblast-dependent bone loss, and perhaps other oxidative stress associated processes mediating bone resorption. Supported in part by R01 AA018282 (M.J.R.).
In contrast, inhibitors of thioredoxin reductase, auranofin and 1-chloro-2,4-dinitrobenzene, attenuated H2O2 removal rates in mitochondria by over 70%. Furthermore, oxidation of thioredoxin-2 via arsenic resulted in a significant decrease in H2O2 removal whereas copper-induced glutathione oxidation showed minimal effects. Inhibition of the thioredoxin system also exacerbated mitochondrial H2O2 production by oxidative-stress inducing agents such as paraquat. These data suggest that the thioredoxin/peroxiredoxin system is the major contributor to respiration-dependent H2O2 removal in brain mitochondria. Additionally, mitochondria pre-incubated with paraquat showed severely compromised H2O2 removal rates (up to 80% decrease) and inhibition of thioredoxin reductase activity in a concentration-dependent manner, suggesting dysfunction of the thioredoxin system in response to environmental neurotoxicants. Therefore, in addition to their well recognized role in the production of ROS, mitochondria may also participate in cell signaling events and/or pathological processes by serving as a potent net ROS removal system.

**ROLE OF CYTOCHROME P450 REDUCTASE IN MEDIATING REDOX CYCLING OF 9,10-PHENANTHRENEQUINONE.**

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Among the most toxic components of diesel exhaust particles is 9,10-phenanthrenequinone (PAQ), a known redox cycling agent. During redox cycling, PAQ undergoes an enzymatic (NADPH-dependent one-electron reduction to a semi-quinone radical. Reaction of this radical with oxygen generates reactive oxygen intermediates (ROI). We found that recombinant NADPH-cytochrome P450 reductase (Supersomes™) readily mediates PAQ (0.1-30 μM) redox cycling and generation of ROI, including hydrogen peroxide (Km = 1 μM, Vmax = 282 pmol/min/ml, micromolar protein/min), and in the presence of redox active iron, hydroxyl radicals. PAQ was also found to stimulate the production of ROI by Chinese hamster ovary (CHO) cells. Lysates from CHO wild type cells (CHO WT) and cells overexpressing cytochrome P450 reductase (CHO OR) both generated hydrogen peroxide. (The Km and Vmax were respectively 0.52 μM and 3.1 pmol/unit activity/min for CHO WT cells and 0.32 μM and 34.3 pmol/unit activity/min for CHO OR cells). Using self-referencing microelectrodes, we found that PAQ redox cycling in intact cells was associated with increased oxygen uptake and cellular release of hydrogen peroxide. Similar amounts of hydrogen peroxide were released by both cell types. Taken together, these data indicate that cytochrome P450 reductase can mediate PAQ redox cycling; however, similarities between the CHO WT and CHO OR cells indicate that both cell types have adequate ROI detoxification despite differences in their cytochrome P450 reductase activity. Supported by NIH grants AR055073, ES004738, CA100994, CA057978, and ES05022.

**REGULATION OF INFLAMMATORY RESPONSES: POSSIBLE REDOX SYNPASE BETWEEN NEUTROPHILS AND MACROPHAGES.**

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The term ‘phagocytic synapse’ describes the interaction between the professional phagocytic cell and its target via a complex system of receptors, bridging molecules and ‘eat me’ signals. We hypothesized that the phagocytic synapse is not merely a cell-cell interaction, but also a redox synapse. We predict that the apopotic neutrophils activate macrophages by PS externalization to utilize them as donors of ROS and ROS-derived reactive oxygen species. We found that when macrophages were exposed to thioredoxin-peroxiredoxin-reacted neutrophils, the extracellular thioredoxin levels were increased and the intracellular thioredoxin levels were decreased. In addition, we observed increased H2O2 levels in the extracellular medium of thioredoxin-peroxiredoxin-reacted neutrophils. These preliminary data suggest that oxidative stress may regulate choline transport by choroid plexus. Possible modulation of stress regulation of transport by antioxidants and the essential metal zinc is under investigation.

**TREATMENT WITH AN ETC COMPLEX III INHIBITOR STIMULATES APICAL CHOLINE TRANSPORT IN PRIMARY CULTURES OF CHOROID PLEXUS.**

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This laboratory reported for both intact choroid plexus and primary cultures that low concentrations of cadmium elicited significant increases in the apical transport of the nutritive organic cation choline. In cadmium-treated primary cultures of choroid plexus, the antioxidant N-acetyl cysteine (NAC) attenuated induction of oxidative stress and stress proteins, as would be expected. However, NAC also attenuated up-regulation of choline transport by cadmium, suggesting that the induction of oxidative stress was requisite to the observed increases in choline transport. As first approach to investigate regulation of choline transport in response to oxidative stress in the absence of heavy metals, we examined regulation of apical 3H-choline transport by antimycin-A that, like cadmium, inhibits complex III of the electron transport chain (ETC). Primary cultures of choroid plexus were treated with 5 μM antimycin-A for 6 h. The mitochondrial membrane potential was monitored using the potential-sensitive fluorescent probe TMRE and epi-fluorescence microscopy; antimycin-A reduced fluorescence of TMRE, indicating depolarization of mitochondrial membrane potential. The ETC inhibitor also increased total cellular accumulation of reactive oxygen species (ROS), as per increased fluorescence in cells loaded with fluorescein diacetate. Increased ROS and mitochondrial membrane depolarization were consistent with induction of oxidative stress. Thus, 30-minute apical uptake of 10 μM 3H-choline then was assayed in the absence of the ETC inhibitor and compared to non-treated and antimycin-A-treated cells. As compared to controls, 3H-choline uptake was stimulated by nearly 60% following antimycin-A treatment. These preliminary data suggest that oxidative stress may regulate choline transport by choroid plexus. Possible modulation of stress regulation of transport by antioxidants and the essential metal zinc is under investigation.

**HIGH SATURATED-FAT DIET AND DEFICIENT NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE ARE CONTRIBUTING FACTORS TO MITOCHONDRIAL DISFUNCTION IN C. ELEGANS.**

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Type 2 diabetes is a worldwide epidemic affecting over 246 million people, but the cellular mechanisms that initiate the disease are still unclear. Recent research indicates that there may be a link between mitochondrial dysfunction and decreased insulin, which is a risk factor for the development of type 2 diabetes. The control of insulin secretion is dependent on the energy state of the pancreatic beta cells making it susceptible to disruption of mitochondrial function and ATP levels. The aim of this work was to investigate the role of a mitochondrial enzyme, Nicotinamide Nucleotide Transhydrogenase (NNT), in maintaining mitochondrial function in animals exposed to high-fat diets enriched with stearic, oleic or linoleic acid. These studies were performed using the nematode, Caenorhabditis elegans, and included the examination of both wild-type nematodes and two different deletion strains lacking the C. elegans ortholog of NNT, nnt-1. The different strains were grown on high-fat and normal diets and the function of mitochondria in these nematodes was examined using oxygen consumption to measure respiratory rate and fluorescent imaging techniques to indicate mitochondrial reactive oxygen species (ROS) production and membrane potential. Our results show that, when grown on normal diets, the nnt-1 mutant nematodes had lower respiratory rates and lower mitochondrial membrane potentials as compared to wild type, suggesting a defect in mitochondrial function. In addition, our results show increased mitochondrial dysfunction and reactive oxygen species production in nematodes grown on a high saturated-fat diet as compared to normal and unsaturated-fat diets. The results suggest that NNT plays a role in maintaining mitochondrial respiratory function and that a high saturated-fat diet increases mitochondrial dysfunction via increased ROS production and decreased energy production.
Oxidative stress plays a key role in acrylonitrile (AN) neurotoxicity. Accordingly, it is important to address the efficacy of antioxidants in protecting against AN-induced free radical damage. This study tested the hypothesis that pretreatment with neonatal rat cortical astrocytes with curcumin protects against oxidative damage caused by AN, addressing potential mechanisms that are involved in activation of the Nrf2 pathway and resultant induction of phase II detoxification enzymes as well as antioxidant enzyme gene expression. Cortical astrocytes were pretreated with 2, 5, 10, 20 μM Curcumin (CUR) 6 h prior to AN treatment (1 mM for 12 h). Pretreatments with CUR significantly increased cell viability and reduced cytotoxicity, compared with astrocytes treated with AN alone. Analysis of markers of oxidative stress and immunocytochemical detection of Nrf2 nuclear translocation demonstrated that pretreatment with CUR increased expression of HO-1 and gamma-GCS genes in the downstream of Nrf2. These results suggest that CUR stimulates Nrf2 activation and increased expressions of HO-1 and gamma-GCS genes in the downstream of Nrf2, thus offering a novel therapeutic modality to attenuate oxidative stress produced by AN.

LYSOSOMAL IRON RELEASE ENHANCES CELL KILLING AFTER PHOTODYNAMIC THERAPY MEDIATED BY A MITOCHONDRIA-TARGETED PHOTOSENSITIZER IN CANCER CELLS.

In photodynamic therapy (PDT), light activates a photosensitizing drug added to a tissue, resulting in singlet oxygen formation and cell death. The photosensitizer, phthalocyanine 4 (Pc 4), localizes primarily to mitochondrial membranes in cancer cells, resulting in mitochondria-mediated cell death. Another Pc 4 derivative, Pc 181, accumulates into lysosomes. In comparison to Pc 4, Pc 181 is a more effective photosensitizer at promoting killing cancer cells after PDT. To assess further how lysosomes contribute to PDT, we monitored cell killing of A431 cells after PDT with Pc 4 and Pc 181. Ammonium ferric citrate (0-30 μM) for 48 hours prior to PDT. Ammonium ferric citrate greatly enhanced Pc 4 plus bafilomycin-induced cell killing without having toxicity by itself, indicating that increasing the amount of chelatable iron stored in the lysosomes enhances the efficacy of bafilomycin-mediated PDT. The iron chelators, desferal and starch-desferal, and the inhibitor of mitochondrial calcium (and ferrous iron) unipporter, Rsu360, protected against Pc 4 plus bafilomycin toxicity. These results support the hypothesis that lysosomal disruption can augment PDT with Pc 4, which targets predominantly mitochondria, but less so after PDT with Pc 181, since Pc 181 already targets lysosomes. Therefore, agents that disturb lysosomal function could potentially be used as an adjuvant treatment with mitochondria-targeted photosensitizers. Supported by CA19079.

NRF2-2 NULL MICE ARE MORE SUSCEPTIBLE TO 1-BROMOPROPAINE-INDUCED HEPATOTOXICITY.

Objectives: 1-Bromopropane (1BP) was introduced as an alternative to ozone-depleting solvents in the workplace. It was found that 1BP exhibits neurotoxicity, reproductive toxicity and hepatotoxicity in rodents and recent occupationally intoxicated cases revealed neurotoxicity of 1BP in humans. However, the mechanism underlying the toxicities of 1BP has not yet been elucidated. The present study investigated involvement of oxidative stress in 1BP hepatotoxicity using nuclear factor erythroid 2-related factor 2(Nrf2) null mice. Methods: Each of 24 male mice of Nrf2-null and wild-type (WT) C57BL/6J were divided into three groups of eight each and exposed to 1BP at 0, 100 and 300 ppm for 8 h/day for 28 days by inhalation. At the end of the exposure, the liver was dissected out immediately. A part of liver was fixed with neutral buffered formalin for histopathological studies and the remaining parts were frozen for biochemical studies. Results: Liver histopathology showed a significantly larger area of liver necrosis in Nrf2-null mice than WT mice. Nrf2-null mice showed higher malondialdehyde (MDA) levels and higher ratios of GSSG/GSH as well as lower total glutathione content, GPx activity and GST activity in the liver of Nrf2-null mice as compared with WT mice. Exposure to 1BP at 300 ppm increased the mRNA expression of HO-1, GST Yc2 and NQO1 in WT mice, but did not influence it in Nrf2-null mice except GST Yc2. Conclusion: Nrf2-2 null mice showed higher susceptibility in the liver to 1BP exposure, being accompanied by higher oxidative stress in the liver. The latter may be through lower expression of anti-oxidative bio-molecules in Nrf-2 null mice. The results are consistent with the oxidative stress contributing to 1BP hepatotoxicity.

NRF2 PROTECTS AGAINST DIQUAT-INDUCED TOXICITY.

Diquat is a contact herbicide that generates superoxide anions through redox cycling. Nrf2 is a transcription factor that up-regulates cytoprotective genes in response to oxidative stress and electrophilic stimuli. To investigate the protective effect of Nrf2 against diquat-induced toxicity, wild-type (WT), Nrf2-null, and Keap1-knock down (Keap1-kd) mice (with enhanced Nrf2 activity) were treated with diquat (125 mg/kg, i.p.) 3 hr after exposure to 1BP. Blood and tissues were collected 1, 2, 4, and 6 h thereafter. Relative GST mRNA level in the liver was increased by 2 fold. Treatment with Keap1-kd mice had attenuated lung edema and no apparent histopathological changes. At the end of the exposure, the liver was dissected out immediately. A part of liver was fixed with neutral buffered formalin for histopathological studies and the remaining parts were frozen for biochemical studies. Results: Liver histopathology showed a significantly larger area of liver necrosis in Nrf2-null mice than WT mice. Nrf2-null mice showed higher malondialdehyde (MDA) levels and higher ratios of GSSG/GSH as well as lower total glutathione content, GPx activity and GST activity in the liver of Nrf2-null mice as compared with WT mice. Exposure to 1BP at 300 ppm increased the mRNA expression of HO-1, GST Yc2 and NQO1 in WT mice, but did not influence it in Nrf2-null mice except GST Yc2. Conclusion: Nrf2-2 null mice showed higher susceptibility in the liver to 1BP exposure, being accompanied by higher oxidative stress in the liver. The latter may be through lower expression of anti-oxidative bio-molecules in Nrf-2 null mice. The results are consistent with the oxidative stress contributing to 1BP hepatotoxicity.
mice; the decrease was more pronounced in Nr2f-null and less in Keap1-kd mice. After diquat treatment, the mRNA of the GSH synthesis enzyme Gclc was increased in Keap1-kd, but not in Nr2f-null mice. In conclusion, Nr2f activation lowers lipid peroxidation products in the circulation and prevents liver and lung injury from diquat, likely due to higher concentrations of GSH, and induction of genes involved in detoxication. (Supported by NIH grants ES009649, ES013774, ES009716, ES007079, and RR021940)

The yeast Saccharomyces cerevisiae transcription factor YAP1 mediates an adaptive response to oxidative stress by inducing the expression of a large number of antioxidant genes. When yeast cells are pretreated with a sublethal dose of an oxidant, Yap1 translocates from the cytosol into the nucleus and confers the cells with a resistance against a later challenge from an oxidant at higher concentrations. There are two known mechanisms by which Yap1 may be activated. H2O2 activates Yap1 through a Gpx3-dependent pathway, involving the formation of disulfide bonds between cysteines in the N-terminal and C-terminal cysteine rich domains (CRDs) of Yap1, whereas the thiol-reactive N-ethylmaleimide (NEM) activates Yap1 via a Gpx3-independent mechanism that requires only the C-CRD cysteines of Yap1. In this study, we demonstrate that H2O2 and NEM show no cross protection to each other. Another thiol-reactive chemical, acrolein, also induces a Yap1-mediated adaptive response and can cross protect against NEM, but not H2O2. Cellular localization and functional experiments using yeast strains with mutant Yap1 genes fused to the green fluorescence protein gene indicate that NEM and acrolein activate Yap1 through the same mechanism requiring two of the C-CRD cysteines. By microarray analysis we identify two sets of Yap1-dependent genes that respond specifically to H2O2 or to NEM and acrolein. By functional analysis using yeast single deletion strains we identify genes in each set that provide resistance against the corresponding inducers. These data demonstrate that Yap1 is sensitive to different types of oxidative stress through distinct CRD-mediated mechanisms and responds accordingly with selective expression of protective genes.

In 2008 California experienced a major outbreak of wildfires with transport of smoke over large distances, especially in the Central Valley. Coarse (PM2.5-10) and fine (PM2.5) particulate matter (PM) concentrations were greatly in excess of the standards, consistent with the neutrophilic inflammatory response observed 24 hours after PM administration. Chemical analysis of the PM preparations resulted in relative low polycyclic aromatic hydrocarbons (PAHs) content as compared to published results from typical urban PM. The coarse PM fraction is more active on an equal dose basis than the fine PM despite its lower content of PAHs. We did not find any correlation between the content of any specific PAH (or total PAH content) in the PM fraction and PM toxicity. Concentrations of the oxidation products of phenanthrene and anthracene, phenanthraquinone and anthraquinone, were several-fold higher in the coarse than the fine fraction, suggesting a significant role for atmospheric photochemistry in the formation of secondary pollutants in the wildfire PM and the possibility that such secondary pollutants could be important sources of toxicity in the wildfire PM. We now demonstrate that wildfire PM also causes major increases in oxidative stress in mouse lungs as measured by decreased antioxidant content in lung lavage fluid.

Aniline exposure causes toxicity to the spleen which is characterized by vascular congestion, hyperplasia, fibrosis and development of a variety of sarcomas in rats. However, underlying mechanisms by which aniline elicits splenotoxic response are not well understood. Previously we have shown that aniline exposure causes oxidative damage to the spleen. To further explore the oxidative mechanism of aniline toxicity, we evaluated the potential contribution of heme oxygenase-1 (HO-1), which catalyzes heme degradation and releases free iron. Male SD rats were given 1 mmol/kg/day aniline in water by gavage for 1, 4, or 7 days, while respective controls received water only. Aniline exposure led to significant increases in HO-1 mRNA expression in the spleen (2- and 2.4-fold at days 4 and 7, respectively) with corresponding increases in protein expression, as confirmed by ELISA and Western blot analyses. Furthermore, immunohistochemical assessment of spleen showed stronger immunostaining for HO-1 in the spleens of rats treated for 7 days, confined mainly to the red pulp areas. No changes were observed in mRNA and protein levels of HO-1 following 1 day exposure. The increase in HO-1 expression was associated with increases in total iron (2.4- and 2.7-fold), free iron (1.9- and 3.5-fold), and ferritin levels (1.9- and 2.1-fold) at 4 and 7 days of aniline exposure. Our data suggest that HO-1 up-regulation in aniline-induced splenic toxicity could be a contributing pro-oxidant mechanism, mediated through iron release, and leading to oxidative damage. Supported by ES04676.

Nitrosamines and nitrosamides, two classes of N-nitroso compounds (NOC), may be implicated in human colon carcinogenesis following gastro-intestinal nitration processes. Since genotoxic concentrations of nitrosamines and nitrosamides were previously reported to result in distinct effects on gene expression after induction in colon cells in vitro, in particular pathways involved in oxidative stress, we hypothesized that differences in radical generation are responsible for these discriminating transcriptomic responses. To investigate the radical generating capacity of NOC in a cellular system, the human colon adenocarcinoma cell line Caco-2 was exposed to genotoxic levels of HO-1 inducing diquat, diquat DH, and diquat-TG, a nitrosoguanidine (MNNG, 1 μM) and N-methyl-N-nitrosoureia (MNU, 1 mM), and the nitrosamines, N-nitrosodietilhydramine (NDEA, 50mM), N-nitrosodiethyljhydramine (NDMA, 100 μM), N-nitrosopiperdine (NPIP, 40 μM), and N-nitrosopyrrolidine (NPPY, 100mM) for 30 minutes and measured by ESR spectroscopy. Nitrosamine exposure resulted in the formation of reactive oxygen species (ROS) and a carbon centered radical, identified as the α-α-nitrosoamine radical, which was catalyzed by the presence of cells, thus suggesting the need for metabolic activity. MNU exposure resulted in a small ROS signal, and formation of a nitrogen centered radical (NCR), the amidyl radical, also catalyzed by presence of cells. MNNG did not influence radical formation, but at a concentration of 1mM, exposure resulted in the formation of both ROS, as well as, in NCR formation. Nitrosamines no longer displayed any radical formation at this concentration. By associating gene expression patterns with ROS formation, we identified several cellular processes including apoptosis, cell cycle blockage, DNA repair and oxidative stress. Cellular processes were only affected by nitrosamines, analogous to the difference in ROS expression patterns with ROS formation, we identified several cellular processes including apoptosis, cell cycle blockage, DNA repair and oxidative stress. Cellular processes were only affected by nitrosamines, analogous to the difference in ROS levels, suggesting that ROS formation plays an important role in the gene expression effects following NOC exposure in Caco-2 cells.

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reactivity of Cys>>His/Lys. GSH conjugation and transporter-mediated efflux is the major pathway of 4-HNE detoxification. This results in depletion of cellular GSH stores and restoration of GSH homeostasis requires de novo GSH biosynthesis. The first and rate-limiting step in GSH biosynthesis is catalyzed by glutamate cysteine ligase (GCL), a heterodimeric holoenzyme composed of a catalytic (GCLC) and modulatory (GCLM) subunit. The relative levels of the GCL subunits are a major determinant of cellular GSH biosynthetic capacity and 4-HNE induces the expression of both GCL subunits as an adaptive response to oxidative stress.

In this study, we have identified several Cys residues adducted by 4-HNE that may be functionally relevant based on in silico molecular modeling. In aggregate, these findings demonstrate that 4-HNE alters GCL holoenzyme formation and activity via direct post-translational modification of the GCL subunits in vitro. Within a cellular context, this novel post-translational regulation of GCL activity could significantly affect cellular GSH homeostasis and GSH-dependent detoxification during periods of oxidative stress.

Sulfur trafficking pathways are highly conserved, complex protein machineries that are responsible for extracting sulfur from cysteine and synthesizing the sulfur-containing cofactors and thionucleosides. The universal sulfur donor in these pathways is the persulfide intermediate, in which a sulfur atom is transiently and covalently bound to a protein cysteine sulhydryl. Despite the biological importance of sulfur-containing molecules, sulfur trafficking pathways are poorly understood. We have developed a method for the in vivo identification of protein interactions within sulfur trafficking pathways and have applied it to the SUF and CSD Fe-S cluster biosynthesis systems of E. coli. The SUF system serves to synthesize Fe-S clusters during the potentially cytotoxic conditions of oxidative stress and iron limitation, whereas the function of the CSD system is unknown. Protein interactions within E. coli expressing an affinity-tagged protein of interest are trapped with acid, and the proteins that copurify with the protein of interest are identified by mass spectrometry. Using this method, we have obtained in vivo confirmation of protein interactions that were established in vitro and identified potentially novel interactions. We have confirmed that SufE interacts with both SufS and SufB, and that CsdE interacts with CsdA. Our results also suggest that both SufE and CsdE interact with the tryptophanase enzyme, which metabolizes cysteine, and thus we propose that tryptophanase may represent an alternative source of sulfur for the SUF and CSD pathways.

The influence of aging on susceptibility to environmental contaminants is poorly understood. The objectives of this study were to test whether oxidative stress (OS) is a potential toxicity pathway following toluene exposure and to determine if these effects are age-dependent. We have used a microarray oxidative stress microarray to investigate the production of reactive oxygen species (NADPH Quinone oxidoreductase 1 (NQO1), NADH Ubiquinone reductase (UBIQ)), antioxidant homeostasis [total antioxidant substances (TAS) and glutathione metabolism (SOD, GCS, GST, GPX and GRD)], and oxidative damage (total aconitate and protein carbonyls). Male Brown Norway rats (4, 12, and 24 mos) were dosed orally with toluene (0, 0.65 or 1 g/kg) in corn oil. Four hours later, frontal cortex (FC), cerebellum (Cb), striatum (Str), and hippocampus (Hip) were dissected out, quick frozen, and stored at 80°C until analysis. Results indicated constitutive age-related changes in some parameters in selected brain regions (eg NQO1 in Cb and TAS in Str). Toluene effects on several OS endpoints were age- and brain region-specific. For example, tolune exposure increased NQO1 activity at 4 and 12 mos in FC and Cb but only at 24 mos in Hip. In contrast, tolune decreased TAS levels at 4 mos in all brain regions and at 24 mos in Cb, but increased TAS levels at 12 and 24 mos in FC, Cb, and Hip. Toluene effects on glutathione enzymes were also age- and brain region-specific.

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Markers of oxidative damage reached significance only at selected ages and/or doses. Aconitase levels were increased in 12 mos FC at 0.65 and 1.0 g/kg, and 24 mos in FC at 0.65 g/kg, and 24 mos in Cb at 1.0 g/kg toluene. Significant increases in protein carbonyl levels in both FC and Cb matched the pattern of aconitase in the FC. These results indicate OS as a potential toxicity pathway, but the complex interaction between age and toluene exposure on OS parameters in brain needs further evaluation. (This abstract does not necessarily reflect U.S. EPA policy).

**784** CHRONIC HIPPOCAMPAL MITOCHONDRIAL OXIDATIVE AND NITROSATIVE STRESS FOLLOWING KAINATE ADMINISTRATION.

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Kainate is a chemoconvulsant which causes excitotoxic brain damage and chronic seizures. The development of chronic epilepsy weeks after acute administration of kainate is known as epileptogenesis. Work in our laboratory has shown increased mitochondrial oxidative stress in the rat hippocampus within hours and days after systemic administration of kainate. The goal of this study was to determine indices of oxidative and nitrosative stress during kainate-induced epileptogenesis. Rats were injected with a single high dose of kainate or vehicle and monitored for seizure activity for 6 weeks. Evidence of reactive species damage was measured acutely after kainate administration, days after kainate prior to development of epilepsy (i.e. latent period) and during the chronic stages of epilepsy. A 40-50% decrease in hippocampal GSH/GSSG and cysteine/cystine redox ratios as well as elevated reactive nitrogen species (RNS) was observed shortly after kainate injection (8h-48h). A 20% increase in nitric oxide production and 10-fold increase in the 3-nitrotyrosine ratio was observed. The mitochondrial redox status measured by reduced coenzyme A and its disulfide with glutathione (CoASH/CoASSG) was decreased nearly 80% shortly after kainate injection and remained permanently decreased for the 6-week duration. The time-dependent profiles of increased oxidative/nitrosative species further correlated with indices of mitochondrial dysfunction such as complex I inhibition and ATP depletion. A decrease in activity of the energy dependent Na+-K+ ATPase was also observed after kainate treatment linking mitochondrial dysfunction with neuronal excitability. The data suggest that prolonged production of ROS/RNS permanently alters the mitochondrial redox status and results in loss of mitochondrial function. Persistent loss of mitochondrial function may be a long lasting consequence of chemoconvulsants such as kainate. Supported by RO1NS039587 (MP)

**785** HAART DRUGS INDUCE OXIDATIVE STRESS IN BLOOD BRAIN BARRIER- ROLE IN HIV ASSOCIATED DEMENTIA.

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The era of highly active antiretroviral therapy (HAART) has led to a considerable decline in the incidence of HIV associated dementia (HAD), but there has been a significant increase in clinical descriptions of minor neurocognitive disorders in HAART treated subjects who live longer. Blood brain barrier (BBB), a tight joining of the endothelial cells of the capillaries surrounding the brain regulates the flow of substances, thereby protecting the brain. Disruption of BBB has been known to play a major role in these neurocognitive disorders. The current study was undertaken to investigate the effect of two HAART drugs namely 3-Azido-3-deoxythymidine (AZT) and Indinavir (IDV), on brain endothelial cells and whether HAART-induced endothelial dysfunction was mediated by oxidative stress mechanism. Human brain microvascular endothelial cells (HBMECs) treated with these drugs alone and in combination produced significant decrease in viability at very low concentrations, 50μM - 200 μM. Increase in reactive oxygen species (ROS) production, as well as mitochondrial dysfunction, was observed, as measured by changes in mitochondrial membrane potential and free cytosolic calcium (Ca2+) levels. Various oxidative stress parameters, like glutathione (GSH) and malondialdehyde (MDA) levels were also determined. Endothelial cells exposed to drugs showed a dose dependent decrease in GSH levels and increase in lipid peroxidation, indicating that cells were undergoing oxidative stress. Transient electrical resistance (TER) measurement and dextran permeability assay, results indicate that HAART drugs can alter the structural integrity and permeability of blood brain barrier. Our data suggest that, in brain endothelial cells, HAART drugs induce mitochondrial dysfunction with simultaneous increase in oxidative stress and may contribute to HIV associated cognitive disorders.

**PS 786** ZINC DEFICIENCY CONTRIBUTES TO ALCOHOL-INDUCED INTESTINAL BARRIER DISRUPTION: ROLE OF HNF-4α

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Intestinal barrier disruption is a causal factor in the development of alcoholic endotoxemia, but the mechanisms have not been fully defined. Zinc is a micronutrient that is required for maintaining normal function of mucosa, and zinc deficiency has been documented in alcoholic liver disease. The present study was undertaken to determine (1) if alcohol affects intestinal zinc homeostasis, and (2) how zinc links with the intestinal barrier structure and function. Mice were pair-fed liquid diet containing ethanol or isocaloric maltose dextrose for 4 weeks. Alcohol exposure caused endotoxemia and hepatitis as indicated by elevated blood endotoxin levels and ALT activities and neutrophil infiltration in the liver. Alcohol exposure remarkably increased the ileal permeability to FITC-dextran. Immunofluorescence detected a decrease in tight junction proteins in the ileum. Alcohol exposure increased reactive oxygen species, but decreased the zinc level in the ileum. Hepatocyte nuclear factor-4α (HNF-4α), a zinc finger transcription factor, were also reduced by alcohol exposure. The link between zinc and HNF-4α in epithelial barrier function were studied in Caco-2 cell culture. Zinc mobilization due to oxidative stress was associated with alcohol-induced epithelial barrier disruption. Experimental zinc deprivation caused disassembly of tight junction proteins and disruption of the epithelial barrier in association with HNF-4α dysfunction. Zinc deprivation also exaggerated the deleterious effects of alcohol. To define the role of HNF-4α in regulation of epithelial barrier, HNF-4α siRNA transfection was performed. HNF-4α siRNA transfection resulted in disassembly of junction proteins and disruption of the epithelial barrier. These results suggest that zinc deficiency is involved in the development of alcohol-induced intestinal barrier disruption, and inactivation of HNF-4α is a potential mechanism underlying the deleterious effects of zinc deficiency on the epithelial barrier.

**PS 787** INVOLVEMENT OF LIVER FATTY ACID BINDING PROTEIN IN LIPID ACCUMULATION CHARACTERISTIC TO ALCOHOLIC LIVER DISEASE.

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Chronic ethanol consumption is a prominent cause of liver disease and is responsible for significant morbidity and mortality throughout the Western world. Among several histologic abnormalities resulting from ethanol ingestion, the prominent pathology is hepatosteatosis (fatty liver). It is hypothesized that the accumulation of lipid associated with chronic ethanol consumption is attributable to the covalent modification of liver fatty acid binding protein (L-FABP) by the aldehyde product of lipid peroxidation, 4-hydroxynonenal (4-HNE). Reactive aldehydes are capable of modifying both proteins and DNA, potentially rendering them inactive. Perturbations in L-FABP function may prove deleterious, resulting in altered intracellular trafficking of hydrophobic ligands throughout cellular compartments. More importantly, it may explain the observed decrease in activation of peroxisome proliferator-activated receptor alpha (PPAR-α); known to be responsible for transcriptional activation of genes involved in β-oxidation. We have observed the covalent modification of L-FABP in vitro as well as in vivo in a rat model of early alcoholic liver disease. Through the use immunoblotting and residue blocking techniques, LC-MS, and MALDI-TOF mass spectrometry, it was determined recombiant L-FABP is covalently modified by 4-HNE. In a rat model of chronic ethanol consumption, a proteomics-based approach identified L-FABP as an immunopositive protein for 4-HNE modification. In addition, the expression of various proteins involved in lipid uptake and trafficking were evaluated, including L-FABP. Collectively, L-FABP may be considered as an important and attractive target for study in the progression of ALD. (Supported by R37 NIH AA009300)

**PS 788** PULMONARY TOXICITY ASSESSMENT OF MULTIWALL CARBON NANOTUBES AFTER SINGLE INTRATRACHEAL INSTILLATION IN RATS.


This study was carried out to assess the pulmonary toxicity of multi-wall carbon nanotubes (MWCNTs) in rats. Purified MWCNTs were instilled intratracheally at dosage of 0.04, 0.2 or 1.0 mg/kg body weight to male Sprague-Dawley rats. Quartz particles in the form of crystalline silica at 5 mg/kg body weight were administered.
in a similar manner. The pulmonary response was characterized by analysis of lung weight, bronchoalveolar lavage fluid (BALF) biomarkers such as the number of white blood cells and neutrophils, lactate dehydrogenase (LDH), protein, and cytokines, and histopathological evaluation of lung tissue at 3 days, 1 week, 4 weeks, and 3 months after a single instillation. In the 1.0 μg/kg MWCNT group, inflammatory responses were observed in the lungs, with increased white blood cells, neutrophils, protein contents in BALF at 3 days after administration. Histopathological examination of the lung, inflammatory cell infiltration were observed up to 1 week after administration in the 1.0 μg/kg MWCNT group. In the 0.6 and 0.2 μg/kg MWCNT group, no evidence of inflammation was observed at any time points after instillation. In the 5 μg/kg quartz particle group, inflammatory response was observed in the lungs, with increased white blood cells, neutrophils, LDH and protein contents in BALF, and histopathological changes in the lung including foamy alveolar macrophage accumulation, inflammatory cell infiltration up to 3 months after administration. These results suggest that MWCNTs induced inflammatory response to the lung in a dose dependent manner. The no-observed adverse-effects level for MWCNT was 0.2 μg/kg in this study.

789 DIFFERENTIAL EFFECTS OF SINGLE-WALLED CARBON NANOTUBES ON HUMAN CARCINOMA CELL LINES.

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Carbon nanotubes (CNTs) are attracting significant attention as a novel material that will play an essential role in future innovations. However, its toxicity is a major concern. Many in vitro studies have assessed the cytotoxicity of CNTs, but the effects differ according to the cell lines and methods used to synthesize CNTs; these differences in cytotoxicity still remain unclear. In this study, the cytotoxic effects of single-walled CNTs (SWNTs) on human lung carcinoma A549 cells and human head and neck carcinoma FaDu cells were investigated. The SWNTs used in this study were manufactured using the arc electrical discharge method. SWNTs were produced with Ni and Y (SO-SWNT), and Fe (FH-P-SWNT) as a catalyst. Cell viability was evaluated by biofilm biomass (crystal violet staining assay) and intracellular metabolic activity (mitochondrial reduction of resazurin and intracellular ATP content). SWNTs were exposed to the cell lines at the concentration up to 1 mg/ml, which was the maximum concentration dispersible in cell culture medium. On 24-h exposure of 1 mg/ml SO-SWNT, the biofilm biomass of the A549 cells was reduced to 26% compared to untreated cells, whereas that of the FaDu cells remained over 90%. On 24-h exposure of 1 mg/ml SO-SWNT to A549 and FaDu cells, the mitochondrial reduction of resazurin decreased to 24% and 37%, and intracellular ATP content decreased to 40% and 54%, respectively. FaDu cells exhibited higher viability values compared to A549 cells in all 3 studies. It was surmised that SO-SWNTs affected cell viability, but the degree of this effect differed between the 2 cell lines. In addition, these nanotubes affected intracellular metabolic activity more than they did the biofilm biomass, even though they were exposed to the cells for a shorter time. We will also present the effects of FH-P-SWNTs on these cell lines in order to clarify the effect of the methods used to synthesize CNTs.

790 EVALUATION OF THE INTERACTIONS BETWEEN MULTI-WALLED CARBON NANOTUBES AND AN IN VITRO MODEL OF THE HUMAN INTESTINE.

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Carbon nanotubes have many current and anticipated applications due to their high strength to mass ratio, their electrical and thermal conductivity, and the ease with which their surfaces can be functionalized. Various studies have demonstrated that multiwalled carbon nanotubes (MWCNTs) can cause oxidative stress, cell death, and genetic damage. Although there are little data on environmental and occupational exposures and exposures to MWCNTs, it is anticipated that exposures will occur as production and applications increase. However, it is not clear whether MWCNTs will be taken up following dermal, inhalation, and ingestion exposure. In this study, the potential for MWCNTs to damage or be taken up following ingestion was assessed using an in vitro model of the human intestine based on the Caco-2 cell culture model. The MWCNTs were well-characterized in terms of size, surface area and surface content. Uptake was examined in MWCNTs for 15 minutes to 24 hours and analyzed by transmission electron microscopy (TEM) and scanning electron microscopy. Cell-to-cell contact was evaluated by immunocytochemistry and cytotoxicity was assessed by measuring LDH release. The TEM images revealed that the MWCNTs aggregated in media and caused damage to the brush border of the Caco-2 cells though cytotoxicity was not observed. There was no evidence that the MWCNTs were taken up by the Caco-2 cells. However, uptake of MWCNTs was observed in a macrophage cell, which was examined as a positive control for uptake. These results suggest that oxidized MWCNTs are not readily taken up by the absorptive enterocytes in the intestine though they might be transported across the intestine through Peyer’s patches.

791 ADVERSE EFFECTS OF FULLERENOL: MITOCHONDRIAL DYSFUNCTION AND CYTOSKELETAL DYSRUPTION IN A RENAL CELL MODEL.

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Fullerenol (hydroxylated fullerene), has been shown in our laboratory to interact with the autophagy pathway and promote non-apoptotic cell death, in the porcine renal proximal tubule cell line (LLC-PK1). Here, we further evaluate the mechanism of fullerenol toxicity in LLC-PK1 cells. In this study, sub-lethal fullerenol treatment (0.63–6.3 mM) promoted dose-dependent ATP depletion, with ATP levels decreased to 50% and 4% of control, 24 and 48 hours post exposure, respectively. Cells treated with the sub-lethal fullerenol concentration were fixed and stained with the cationic dye, Mitotracker Red-CMXRos, demonstrated loss of mitochondrial membrane potential as early as 3 hours post treatment. Remarkably, co-treatment of fullerenol and the autophagy inhibitor, 3-methyl adenosine, restored mitochondrial membrane potential and ameliorated ATP depletion in this cell model. Microscopic studies of fullerenol treated cells stained with the actin binding dye, phalloidin, revealed disruption of actin cytoskeletal protein, 24 hours post exposure, at fullerenol concentrations that correlated with the autophagy response. Evaluation of fullerenol in an acellular actin polymerization assay identified interference in actin polymerization kinetics as a plausible mechanism for the observed fullerenol actin disruption. Other findings included minimal lipid peroxidation 3– 24 hours post fullerenol exposure, as determined by TBARS analysis and BODIPY staining of treated cells. Taken together, the cellular responses to fullerenol treatment in the LLC-PK1 cell model were multi-faceted, and consist of, autophagy activation, reduced mitochondrial function with ATP depletion, cytoskeletal protein disruption, and limited lipid peroxidation at sub-lethal fullerenol concentrations. Further studies are needed to establish if the results obtained are specific to LLC-PK1 renal cells or are indicative of a general cellular response to fullerenol exposure.

792 SAFETY EVALUATION OF FULLERENOL NANOWHISKER AS A NEW NANOPRODUCT - IN VITRO EXPERIMENT.


[Purpose] Needle-like crystals of fullerene developed by Miyazawa et al. (J Mater Res 17:83-88, 2002) is named C₆₀ fullerene nanowhisker (FNW) with an average length of about 6 μm and an average diameter of about 660 nm. Its unique properties can become a promising carbon device. The purpose of this study is to elucidate its mutagenic and cytotoxic activities in vitro using various cell lines. [Methods] FNWs' provided from Dr. Miyazawa and 99.9% pure C₆₀ were used at 0.1, 1.0 and 10 μg/ml dispersed in culture medium. The mutagenecity was evaluated by Ames test using four Salmonella typhimurium and one Escherichia coli strains according to OECD guideline. The cytotoxicity and the rate of cell proliferation were determined by LDH transduction test and MTT assay, respectively. [Results] Neither C₆₀ nor FNWs at concentration between 0.1 – 10 μg/ml were mutagenic in any bacterial strain without metabolic activation. 2. Three macrophage-like cell lines(THP-1, J774.1 and RAW264) phagocytosed FNWs gradually after its addition to the culture medium as well as C₆₀. 3. LDH transduction rate of epithelial cells (A549) and three kinds of macrophage-like cells was less than 5% after 3 day incubations with C₆₀ or FNWs (0.1 – 10 μg/ml), suggesting that C₆₀ and FNW have little or no cytotoxicity. 4. While C₆₀ increased cell proliferation by 1.1 – 1.4 times compared to the control, the presence of FNWs did not affect the level of L929 fibroblast proliferation. [Conclusion] These results suggested that FNWs are safe nanoproduct similar to other C₆₀ in vitro. Okuda et al. (Annual Meeting Japanese Society for Biomaterials, 416, 2007) suggested that FNW may not stress protein HSP70B mRNA in HeLaS3 cells. This result may support our presented results. It will require in vivo experiment for safety evaluation of FNW to the next step.
PULMONARY RESPONSE, OXIDATIVE STRESS AND GENOTOXICITY INDUCED BY CARBON NANOFIBERS.

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Carbon-based nanomaterials are considered to be one of the key elements in nanotechnology. Their structure gives an unusual combination of properties that are highly desirable for many industrial products. High aspect ratio makes them an attractive structural material, but their nanometer-scale diameter and needle-like shape have drawn comparisons with asbestos. It is known that inhaled asbestos fibers induce proliferation of connective tissue (fibrogenic response) and increase the risk of acquiring pulmonary carcinoma. We have previously reported that exposure to fibrous single walled carbon nanotubes (SWCNT) caused a robust, acute inflammation with early onset of interstitial fibrosis, formation of granulomas, K-ras mutations found in mouse lungs and DNA damage observed in V79 cells. In the current study, we compared effects of carbon-based nanofibers (Pyrograf-III) with asbestos fibers (crocidolite) or SWCNT in vivo and in vitro. We found that in vitro exposure of RAW264.7 macrophages to nanofibers caused cytotoxicity and ROS production. Additionally, treatment of V79 cells with nanofibers caused adverse effects examined by two different genotoxicity assays (comet and micronucleus tests). Pulmonary exposure to nanofibers resulted in an augmentation of biomarkers of cell injury and oxidative stress, strong acute inflammation, as well as interstitial fibrosis and increased collagen deposition in mouse lungs. Mice exposed to an equal dose of nanofibers or asbestos induced less collagen deposition as compared to that seen after exposure to SWCNT. Our current results strongly indicate the need for further assessments on the health effects of nanofibers.

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CHARACTERIZATION OF MULTI-WALLED CARBON NANOTUBES (MWNTs).

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The production of engineered multi-walled carbon nanotubes (MWNTs) has increased significantly in recent years. MWNTs exhibit unique physical, chemical, optical, and electrical properties that have made them very attractive candidates for use in composite materials, energy conversion, packaging, consumer healthcare and medical applications. However, there are very limited data available on human exposure to MWNT and the potential health effects associated with exposure. Many of the unique physicochemical properties of MWNTs are known to influence biological activity and may play a significant role in their assessment of toxicity. Six different types of MWNTs were selected for use in the study based on their specified length and diameter. Each MWNT type was obtained from 3-5 sources, respectively. Many of the small diameter nanotubes met their vendor-supplied specifications whereas all longer-tubes did not meet their vendor-supplied specifications.

The diameter and length were determined for samples using HR-TEM and SEM, respectively. Many of the small diameter nanotubes met their vendor provided specifications whereas majority of the medium and large diameter tubes did not. The length of the 2 shortest nanotubes was within their vendor provided specifications whereas all longer-tubes did not meet their vendor-supplied specifications.

MULTI-WALLED CARBON NANOTUBE EXPOSURE INDUCES MAST CELL ACTIVATION AND ALTERS AORTIC VASCULAR REACTIVITY.

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Due to their unique physical and chemical characteristics, the use of nanomaterials has increased dramatically in recent years leading to a growing need for research examining their potential impact on the environment and human health. Multi-walled carbon nanotubes (MWNT) represent an important nanomaterial with wide ranging applications. Mast cells are well recognized for their role in allergy, asthma and cardiovascular disease. The aim of this study was to examine the activation of mast cells in the pulmonary and cardiovascular systems following exposure to MWNTs. We examined the ability of MWNTs to activate bone marrow-derived mast cells (BMMCs) as measured by degranulation and cytokine production. Additionally, we compared the development of pulmonary inflammation and changes in aortic vascular reactivity between C57BL/6 and B6.Cg-Kitw−/− mast cell deficient mice following MWNT instillation. MWNT exposure (10-100 μg/ml) did not alter BM/MC degranulation in vitro, however, MWNTs stimulated production of several pro-inflammatory cytokines. C57BL/6 mice instilled with 10 μg MWNTs developed pulmonary inflammation including increased numbers of macrophages, neutrophils and eosinophils which were associated with increased osteopontin levels. Further, C57BL/6 mice exposed to MWNTs exhibited impaired aortic vascular relaxation to forskolin and altered contractile response to nonepinephrine. These changes in vascular reactivity were not present in B6.Cg-Kitw−/−. These findings demonstrate that MWNT can direct mast cell production of pro-inflammatory mediators that may potentially contribute to impaired vascular relaxation and adverse health effects in vivo. This work supported by NIH ROI1ES5016246 and East Carolina University.

NEUROTOXICITY AND CELLULAR DISTRIBUTION OF SINGLE-WALLED CARBON NANOTUBES.

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Single-walled carbon nanotubes (SWCNT) exhibit unique chemical and physical properties that are attractive for many potential biomedical applications. The evaluation of neurotoxicity of SWCNT is important for realizing these practical applications. However, no neurotoxicity studies of the functionalized materials have been reported. We investigated and compared the concentration-dependent cytotoxicity of SWCNT and functionalized SWCNT with polyethylene glycol (SWCNT-PEG) in PC12 cells using the MTT and LDH assay. SWCNT elicited cytotoxicity in a concentration-dependent manner, and SWCNT-PEG exhibited less potency than SWCNT. Lesser cytotoxicity of SWCNT-PEG suggested that surface properties of carbon nanotubes may contribute to their cytotoxicity. Moreover, reactive oxygen species (ROS) were generated in a concentration-dependent manner after exposure to these nanomaterials, indicating an oxidative stress mechanism. Furthermore, nuclear condensation with Hoechst 33342 staining and time-dependent caspase 3 activation after exposure to SWCNT (10 μg/ml) shows evidence of apoptosis. Interestingly, more apoptotic PC12 cells appear and there is a higher activity of caspase 3 after exposure to SWCNT-PEG, suggesting that surface conjugated PEG may help to trigger apoptosis signaling. The uptake of these carbon materials by PC12 cells were demonstrated using Raman Microscopy. However, dopamine levels in the cells were not significantly altered at 24 hours as measured by HPLC/EC. These studies provide a framework for further characterizing the neurotoxic potential of different types of SWCNT and suggest that the nervous system may be targeted by single-walled carbon nanotubes under some conditions. Support by NCTR E7282 and ORISE.

DISPERSION STATUS OF SINGLE WALLED CARBON NANOTUBES IS A KEY DETERMINANT OF THEIR BIOLOGICAL ACTIVITIES.

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Single walled carbon nanotubes (SWCNT) have wide applications, but raise an urgent concern regarding their potential toxicities. A major obstacle to the biological and toxicological evaluation of nanoparticles is their dispersion in biological samples or buffers. SWCNT tend to form large agglomerates in solutions which affect
their bioactivities. Previous studies have shown that pulmonary exposure to dispersed SWCNT caused a greater interstitial lung fibrosis in mice than the non-dispersed form. In this study, we further investigated the effect of nanoparticle dispersion on cellular activities in vitro using a natural lung surfactant (Survanta®) as a dispersing agent. Human bronchial epithelial BEAS-2B cells and human lung fibroblast CRL-1490 cells were exposed to physiologically relevant concentrations of SWCNT (0.02-0.6 μg/cm²) with or without Survanta® (150 μg/ml) and their effects on cell viability, proliferation, and collagen production were determined by LDH assay, cell counting, and Western blot analysis, respectively. The results showed that: 1) Survanta® was effective in dispersing micron-sized SWCNT agglomerates to nano-sized structures; 2) Survanta®-dispersed SWCNT exhibited a biphasic effect on cells inducing proliferation at low doses and causing toxicity at high doses, while non-dispersed SWCNT had no significant effects; 4) In lung fibroblasts, dispersed SWCNT upregulated collagen expression, whereas non-dispersed SWCNT had a lesser effect. These results are supported by in vivo data and suggest that dispersed SWCNT is more fibrogenic than non-dispersed SWCNT. Due to the rapidity and simplicity of the in vitro assay models described in this study, this model could potentially be used as a rapid screening tool for fibrogenicity and toxicity testing of nanoparticles.

**798 GRAPHENE INDUCED CYTOTOXICITY AND OXIDATIVE STRESS: AN IN VITRO STUDY.**

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The unique structure and surface properties of graphene make this carbon-based nanomaterial a good candidate for mammalian and microbial detection. Little is known about the potential toxic effects of this nanomaterial. Therefore, we determined if graphene produces toxicity in PC12 cells. Graphene was characterized using Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and Confocal Raman Microscopy. High-resolution TEM images revealed that the graphene samples are composed of graphene sheets overlaid side-by-side (1-5 layers), with dimensions of 100 nm in diameter. Graphene produces reactive oxygen species (ROS) in a concentration-dependent manner. Graphene toxicity at high doses, while non-dispersed SWCNT had no significant effects; 4) In lung fibroblasts, dispersed SWCNT upregulated collagen expression, whereas non-dispersed SWCNT had a lesser effect. These results are supported by in vivo data and suggest that dispersed SWCNT is more fibrogenic than non-dispersed SWCNT. Due to the rapidity and simplicity of the in vitro assay models described in this study, this model could potentially be used as a rapid screening tool for fibrogenicity and toxicity testing of nanoparticles.

**799 INDUCTION OF MITOTIC SPINDLE ABERRATIONS BY OCCUPATIONALLY RELEVANT DOSES OF SINGLE-WALLED CARBON NANOTUBES.**

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Engineered carbon nanotubes are newly emerging manufactured particles with potential applications in electronics, computers, aerospace and medicine. The low density and small size of these biologically persistent particles makes respiratory exposures to workers likely during the production or use of commercial products. We have previously shown mitotic spindle aberrations in cultured primary and immortalized human airway epithelial cells exposed to 96, 48 and 24 micrograms/cemtimeter squared single-walled carbon nanotubes (SWCNT). To investigate mitotic spindle aberrations at concentrations anticipated in exposed workers, primary and immortalized human airway epithelial cells were exposed to SWCNT for 24-72 hours. We have now demonstrated fragmented centrosomes, multiple mitotic spindle poles and aneuploid chromosome number at doses equivalent to the permissible exposure limit. The nanotube bundles are similar to the size of microtubules that form the mitotic spindle and may be incorporated into the mitotic spindle apparatus. Confocal microscopy demonstrated nanotubes within the nucleus and in association with mitotic tubulin, the centrosomes and the chromatin in cells exposed to 2.4, 0.24 and 0.024 micrograms/cemtimeter squared SWCNT. The lower doses do not cause toxicity by Alamar Blue assay, apoptosis by TUNEL or reduction in colony formation after 24 hours. However, after 3 days, the colony formation of the primary cells was reduced. Our results show significant disruption of the mitotic spindle by SWCNT at occupationally relevant doses. Centrosome fragmentations, mitotic spindle disconnection and aneuploidy are characteristics of cancer cells and may lead to an increased risk of cancer. *The authors contributed equally to the work.

**800 PULMONARY TOXICITY ASSESSMENT OF MULTI-WALLED CARBON NANOTUBES IN VITRO.**

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Multi Walled Carbon nanotubes (MWCNT) are one of the most popular nanomaterials as reflected by their wide range of applications in electronics, material science and biomedical research. However, their fiber-like structure, low specific weight and nanoscale dimension also raises concerns about possible adverse effects on human health and the environment. In this study, physiologically relevant doses (0.06-0.2 μg/cm²) of MWCNT, which have been shown to cause pulmonary disorders in mice, were examined for their cellular toxicity and inflammatory and fibrogenic cytokine production in human bronchial epithelial BEAS-2B cells and alveolar epithelial A549 cells. MWCNT were dispersed in natural lung surfactants and exposed to the cells in culture. Cellular toxicity was assessed over time by direct cell counting and by WST proliferation assay. Inflammatory/fibrogenic TGF-β1 and MMP-9 production was determined by Western blot assay. The results showed that: 1) MWCNT can be effectively dispersed using natural lung surfactants, 2) Dispersed MWCNT caused a substantial decrease in cell viability and proliferation as compared to non-dispersed MWCNT or vehicle control treatment, 3) Dispersed MWCNT also induced TGF-β1 and MMP-9 upregulation in the lung epithelial cells, indicating their fibrogenicity and inflammation potential which is also supported by our in vivo studies. Thus, the in vitro methods may potentially be used as a predictive model for in vivo toxicity assessment and to aid the mechanistic studies of nanomaterial-induced pulmonary toxicities.

**801 CHARACTERIZATION OF THE SURFACE ADSORPTION PROPERTIES OF MULTI-WALLED CARBON NANOTUBES IN BIOLOGICAL CONDITIONS VIA QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR).**

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Carbon nanotubes have been studied extensively for drug delivery and potential toxicity in occupational and environmental exposures. Once entering biological systems, numerous biological components will interact with the surface of carbon nanomaterials. There is no method available to quantitatively measure these interactions. Here, we report a QSAR approach to characterize the surface adsorption properties of multiwalled carbon nanotubes (MWCNT), in which a set of small molecules having diverse physicochemical properties was used as probe compounds. The adsorption coefficients (α) of the probe compounds were obtained by measuring the quantities of the probe compounds adsorbed on the surfaces of the nanomaterials and the equilibrium concentrations of the probe compounds in the media. The log (α) values were scaled to a set of solvation molecular descriptors of the probe compounds via multiple linear regressions to provide a set of five descriptors representing the contributions of the five types of molecular interactions: hydrophobicity (V), hydrogen-bond acidity (α) and basicity (β), dipolarity/polarizability (π), and lone-pair electrons (R). A QSAR model was established for MWCNT using the five nano-descriptors; log (α) = -1.33 + 0.0435R - 1.757α - 0.57α2 - 2.788V + 4.18V2, n = 28, R2 = 0.93, F = 63 with significance of 2.7 x 10-12. The nano-descriptors provide a better correlation (R2 = 0.93) with adsorption coefficients than hydrophobicity (logKow) alone (R2 = 0.57) (Supported by U.S. EPA STAR Grant # R833328 and USAFOSR Grant # FA9550-08-1-0182)

**802 NOVEL MOLECULAR PATHWAYS INDUCED IN FUNCTIONALIZED FULLERENE EXPOSED HUMAN EPIDERMAL KERATINOCYTES AND HUMAN BRONCHIAL EPITHELIAL CELLS.**

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Engineered nanomaterials have been extensively used for diagnostics and therapeutics based on their unique physicochemical properties. Since native C60 is hydrophobic, many hydrophilic C60 fullerenes derivatives have been synthesized to fa-
cilitate clinical and industrial applications. However, the interaction of fullerenes with biological systems is not completely understood. This lack of knowledge limits the development of new fullerene applications in the biological arena, and raises uncertainties about their impact on human health and environment. In the present study, we investigated the bioimpact of three chemically modified fullerene derivatives, hexa carbosyl fullerene adduct (Hexa-C60), tris carbosyl adduct fullerene adduct (Tris-C60) and gamma (γ)-cyclodextrin caged C60 (CD-C60) in both human cutaneous epithelial cells (HEK) and human bronchial epithelial cells (Beas-2B). We evaluate cellular viability, proliferation, and senescence. Our data indicate that all three fullerene derivatives can protect against induced-apoptosis, and that tris-C60 can reduce necrotic cell death in both HEK and Beas-2B cells. In addition, we observed reduced proliferative capacity, reactive oxygen species generation and cytokine production in tris-C60 treated these cells. Moreover, cell morphology and growth characteristics were significantly altered only in the tris-C60 treated cells. Further evaluation of these responses using flow cytometric analysis demonstrated that tris-C60 induced cell cycle arrest at G1 phase. This inhibition of cell cycle progression was not observed in either CD-C60 or Hexa-C60 treated cells. Studies are ongoing to understand the molecular mechanisms underlying these responses.

**803** ENVIRONMENTAL FATE AND EFFECTS OF METALLOFULLERENE SYNTHESIS-GENERATED SOOT IN A TERRESTRIAL SYSTEM.

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Approximately 90% of the carbon that goes into fullerene and carbon nanotubes synthesis results in the formation of amorphous carbonaceous soot. This soot likely contains metals used as catalysts during the synthesis process. The disposal of these soots may not be addressed by current regulations and thus may be disposed of by conventional means (i.e., landfill). This research addresses the acute fate and effects of gadolinium (Gd) metallofullerene generated soot in soil. Individual earthworms (Eisenia fetida; n = 10/treatment) were exposed to a dose-range of metallofullerene soot for 7 and 14 days in soils at pH 5 and 6. Soot contained very high concentrations of Gd (384,500 mg/kg) and copper (Cu; 162,500 mg/kg), as determined by inductively coupled plasma-mass spectrometry. Toxicity increased at soot concentrations > 10,000 mg/kg regardless of pH and acute exposure durations. In contrast, earthworms began to avoid the soils at lower soot soil concentrations, especially at pH 5. Gd and other metals bioaccumulated in earthworms in a dose-dependent manner. Gd and Cu were leached with water from metallofullerene-spiked soils at high concentrations over a 48 h period. In conclusion, these data demonstrate that soil pH does not appear to play a role in metallofullerene soot toxicity in subterranean invertebrates, yet earthworms are likely to avoid soils contaminated with metallofullerene soot at concentrations well below toxic threshold levels. Insight from this research will result in better end-of-life cycle treatment and management of metallofullerene soot, as well as possible remediation strategies should this material spill in the environment.

**804** EFFECT OF CARBON NANOTUBES ON ANTIOXIDANT BALANCE IN THE LUNGS.

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Conventional chemotherapeutic cancer treatments have major limitations and side effects. To decrease the nonspecific toxic effects of these drugs, targeted therapies are being developed, which includes the use of antibodies. However, antibodies do not readily cross the cellular membranes, and need carriers for efficient functioning. Recently, carbon nanotubes (CNTs) are being considered as drug delivery agents due to their unique property to be able to move freely through biological tissues and enter cells. However, CNTs have toxic effects, and surface functionalization of nanotubes has been proposed to reduce the toxicity. In the literature, CNT have been known to induce oxidative stress (OS), but little is known about the mechanism by which these particles induce OS. The objective of this study was to compare the OS-induced toxicity of different types of doublewalled CNTs either in their pristine state (DWildNT-Nanocyl) or functionalized by oxidation (sDWNTs-COOH, CF126) in an in vitro lung model (LA4 cells). LA4 cells exposed to different concentrations of CNTs have a concentration (1,5 and 10 mg/ml) and time dependent effect on cell viability. In addition, cells exposed to non toxic dose of DWNT-Nanocyl and sDWNTs-COOH had significant increase in reactive oxygen species (ROS) and lipid peroxidation products (MDA) as compared to the controls and the CF126 treated cells. A significant decrease in the intracellular glutathione levels were also observed in the cells exposed to DWNT-Nanocyl and sDWNTs-COOH as compared to the control and CF126 treated group, indicating that the cells were undergoing OS induced damage. Based on these data, we can conclude that DWNT-Nanocyl and sDWNTs-COOH were inducing OS by the generation of ROS and alteration of the antioxidant balance in the cells, however, CF126 was the least toxic, and could be a viable candidate for drug delivery agent.

**805** COMPARATIVE CLASTOGENIC STUDY OF FUNCTIONALIZED AND NON-FUNCTIONALIZED MULTI-WALLED CARBON NANOTUBE IN BONE MARROW CELLS OF SWISS-WEBSTER MICE.

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Nanomaterials are part of an industrial revolution to develop lightweight but strong materials for a variety of purposes. Multi-walled carbon nanotubes (MWNT’s) are an important member of this class of materials for their unique physicochemical properties and promises in technological applications. They can be used in sensors, electronic devices, wastewater treatment and many other industrial applications. Importantly, broad biomedical uses of carbon nanotubes such as in drug delivery systems, bone cell growth and cancer treatment have been investigated. With the rapid advances in carbon nanotube-based new materials and technologies, there is a growing recognition that a fundamental understanding of the toxicological properties of carbon nanotubes is imperative. However, the toxicity of carbon nanotubes is barely known when they are introduced into the blood circulation, as a result of their biomedical applications. We have for the first time compared the clastogenic/genotoxic potential of functionalized and non-functionalized MWCNT in bone marrow cells of Swiss-Webster mice; using mitotic index (MI), chromosome aberrations (CA), micronuclei (MN) formation and DNA damage in leukocytes as toxicological endpoints. The results demonstrated that MWCNT exposure significantly increased (p<0.05) the number of structural chromosomal aberrations, the frequency of micro-nucleated cells and the level of DNA damage, and decreased the mitotic index in exposed groups compared to control groups. In summary, purified functionalized MWCNT showed more clastogenic/genotoxic potential than the non-functionalized form and it is shown to be toxic at sufficiently high concentrations. Therefore, careful monitoring with respect to designing/synthesising biocompatible carbon nanomaterials and further characterizing its clastogenicity/genotoxicity in vivo studies is essential.

**806** NEPHSTRIP: A RAPID MUTIPLEX TEST FOR THE SENSITIVE AND SPECIFIC DETECTION OF URINARY KIDNEY INJURY MOLECULE-1 AND CYSTATIN-C.

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Traditional metrics of kidney function (blood urea nitrogen and serum creatinine) are inadequate for the detection of acute kidney injury (AKI). In 2008, in response to the Critical Path Institute’s Predictive Safety Testing Consortium, the United States Food and Drug Administration and European Medicines Agency encouraged the use of seven novel urinary biomarkers, including kidney injury molecule-1 (Kim-1 in rats: KIM-1 in humans) and cystatin-C (Cys-C, for the preclinical evaluation of drug related kidney toxicity. We developed and evaluated a rapid and direct immunochromatographic lateral flow 15-min assay for the simultaneous and sensitive detection of urinary Kim-1/KIM-1 and Cys-C for preclinical (lower limit of detection [LLD]; 0.5 and 78.1 ng/ml for Kim-1 and Cys-C respectively with no cross-reactivity) and clinical (LLD; 0.5 and 31.25 ng/ml with no cross-reactivity) use. In rats exposed to cadmium and doxorubicin, Kim-1 and Cys-C band intensity correlated with histopathological damage, Kim-1 and Cys-C im-munohistochemical staining, as well as Kim-1 and Cys-C levels measured using an established Meso Scale Discovery platform. In human patients at risk for AKI following cardiac surgery, preoperative and postoperative urinary Kim-1 and Cys-C band intensity also correlated with measurement from validated ELISA and Luminex assays. These results indicate that urinary Kim-1/KIM-1 and Cys-C may be simultaneously quantified via a novel rapid dipstick assay. This holds particular promise for the sensitive and specific detection of evolving AKI in the clinical arena, where point of care or home-based testing of patients at risk for AKI is currently unavailable.
Drug-induced kidney injury (DIKI) is not an uncommon finding during non-clinical drug development. Recently, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) accepted more sensitive urinary biomarkers, which are complementary to the current standards for detection of drug-induced acute renal injury in the earlier stages of non-clinical drug development. To determine if significant biological variations in these parameters occur over time using a traditional metabolic cage and the BASi Culex® system, this study investigated baseline levels of eight urinary biomarkers of nephrotoxicity (albumin, t-glutathione S-transferase (tGST), clusterin, lipocalin-2, osteopontin (OPN), kidney injury molecule-1 (Kim-1), GSTYb1, and renal papillary antigen-1 (RPA-1)) using Meso Scale Discovery (MSD) kits and various urinalysis parameters. Briefly, urine samples were collected overnight (16-18 hr) on three occasions (Days 1, 5 and 7) from 24 Han Maleist rats housed in traditional metabolic cages (n = 12) or BASi Culex® automated sampling system with urine collection unit (n = 12). The full panel urinalysis (urine dipstick test, electrolytes and chemistry) results fall within the normal range and the data are comparable between animals from metabolic cage and Culex system on all 3 days. A slightly increased urinary glucose level was noted in Culex system on all these days compared to metabolic cage, but this trend was not statistically significant (P > 0.05). No statistically significant differences (P > 0.05) were found in 7 of 8 urinary biomarkers parameter values between metabolic cage and Culex system on days 1, 3 and 7. Urine GSTYb1 from animals in the Culex system were higher (P < 0.05) than those from metabolic cage on Day 1, but decreased back to levels equal to metabolic cage animals on Days 3 and 7 (P > 0.05). This study provides valuable background information on renal biomarkers of injury in two test systems that may be used in future studies for risk-benefit profiling of pharmaceutical agents.

Establishing the effective nephrotoxicity screening method is very useful to evaluate the possible nephrotoxicants. In vitro cytotoxicity test like as MTT assay, Neutral red assay, LDH assay, is widely used screening method. In our results, newly developed transepithelial electric resistance (TEER) measurement using two-chamber transport system was more sensitive than conventional cytotoxicity test. TEER measurement is very easy and it can be affected earlier and in small dose. So this method can be used as a screening test for nephrotoxicity. Protein expression levels of ZO-1, occludin, claudin-1 didn’t change in cadmium and cyclosporin A treated LLC-PK1 cell. MDA and 8-OHdG levels were increased in cadmium and cyclosporin A treated cell. So oxidative stress may play an important role of nephrotoxic mechanism. In vivo study, urinary N-acetyl-β-D-glucosaminidase (NAG), MDA and 8-OHdG were useful effect biomarker of nephrotoxicants. Urinary NAG were increased at 3 day in cadmium or cyclosporin A treated group. So urinary NAG may be used in toxicologic study field and epidemiologic study to evaluate the nephrotoxicity. Urinary MDA and 8-OHdG were also increased and these indices are correlated with NAG activity. So oxidative stress also play an important role of nephrotoxic mechanism as same as in vitro study result.
tion of p-p38, p-p53, p21 and cyclin B1, as well as partially reversing BrO3- induced G2/M arrest; however, ascorbic acid failed to alter the formation of 8-OHdG induced by BrO3-. On the other hand, N-acetyl-cysteine (NAC), an intracellular precursor of GSH, enhanced the effect of BrO3- on the formation of 8-OHdG. Surprisingly, NAC partially reversed BrO3- induced G2/M arrest. These data support the novel finding that ROS mediated MAPK activation is involved in BrO3- induced cell cycle arrest and that the production of ROS is important of the formation of 8-OHdG. It also supports the hypothesis that GSH plays a dual role in BrO3- induced renal toxicity. This work was supported by Awwa RF 4042, ICA, MWD, NWRI, Calleagas Water, Long Beach Water, SNWA, LADWP, Veolia, Environment Abu Dhabi.

812 In Vivo Disposition and Neophotoxicity of Bromate in F344 Rats.

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The kinetics of bromate absorption and excretion after oral exposures were correlated to renal and thyroid cell proliferation, death and DNA damage to better understand the mode of action of this suspected human carcinogen. Exposure of male and female rats to bromate did not increase bromate blood levels after 28 days compared to controls. In contrast, exposure of male rats to bromate increased bromate in the urine from 26 μg/L in controls, to 4,236 and 20,016 μg/L in rats dosed with 125 and 400 mg/L bromate, respectively. Bromide, a bromate degradation product, increased in the urine of male rats from 58,400 μg/L in controls to 85,522 and 138,590 μg/L after exposure to 125 and 400 mg/L, respectively. Similar results were seen in female rats for both bromate and bromide. Cell proliferation, as assessed by bromodeoxyuridine (BrdU) staining, increased 307 and 437% in male rat kidneys exposed to 125 and 400 mg/L, but only 5 and 19% in female rats kidneys. Analysis of renal cell morphology using H&E staining showed greater increases in glomerular and tubular interstitial proliferation in males exposed to bromate compared to females, which correlated to alterations in 8-OHdG staining, a marker of DNA damage. These data suggest that the disposition and excretion of bromate after oral exposures are similar in male and female rats, which contrasts to its ability to preferentially induce nephrotoxicity in male rats. This is in contrast to bromate’s ability to induce similar levels of thyroid toxicity in male and female rats as determined by H&E and BrdU staining.

813 Antioxidant Defense in Renal Proximal Tubular Cells from Normal and Diabetic Rats.

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Diabetic nephropathy is characterized by increased oxidative stress and altered mitochondrial function. We prepared primary cultures of proximal tubular (PT) cells and mitochondrial fractions from renal cortex from male Sprague-Dawley rats made diabetic with streptozotocin (STZ; 60 mg/kg ip), to determine characteristics of antioxidant defense and susceptibility to chemical injury. PT cells from diabetic rats 30 days after the STZ injection exhibited higher basal and toxicant-stimulated levels of reactive oxygen species, higher mitochondrial membrane potential, and greater susceptibility to chemically induced cytotoxicity as compared to those from age-matched control rats. Both N-acetyl-L-cysteine (NAC) and a cell-permeable catalase derivative (Cat-SKLI) significantly and equally protected PT cells from both normal and diabetic rats. Despite the higher level of basal oxidant stress, mitochondria of PT cells from diabetic rats exhibited 6.7-fold higher content of GSH than those from control rats. We then determined protein expression levels of several key antioxidant systems in renal mitochondria from diabetic rats at both 30 and 90 days post-STZ injection and in those from age-matched control rats. Protein levels of the two mitochondrial GSH transporters were slightly higher in diabetes. While expression of superoxide dismutase 2 (SOD2) was modestly elevated, that of total thiorredoxin-2 (Trx2) was decreased at 30 days and increased at 90 days. Levels of 3-nitrotyrosine-modified proteins in mitochondria were somewhat higher at both times in diabetic rats. Assessment of 4-hydroxy-2-nonenal (HNE)-modified proteins showed primarily 4 bands, which were mostly increased at 30 days but decreased at 90 days. These results show that although a classical antioxidant and GSH precursor, NAC, and a catalase derivative both effectively and equally protect PT cells from both control and diabetic rats from injury, redox processes and mitochondrial energetics are markedly altered in the renal PT cell due to diabetes and change as nephropathy develops.

814 Flow Cytometric Characterization of Mitochondrial Subpopulations in the Kidney.

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Mitochondria in the kidney experience periodic bouts of oxidative stress from metabolic demand and xenobiotic metabolism resulting in persistent damage to mitochondria. We hypothesize that damaged mitochondria accumulate making the kidney more susceptible to failure when stressed by disease or xenobiotic exposure. To assess mitochondrial morphological and functional changes as a result of oxidative stress, flow cytometric techniques have been developed to quantify features of individual mitochondria related to size, calcium concentration, mtDNA content, respiratory capacity and oxidative damage. Mitochondria from rabbit kidneys were stained with molecular probes for cardiolipin content (nonyl acridine orange, NAO) and membrane potential (tetramethylrhodamine, TMRM) and they were analyzed using flow cytometry. Flow cytometric subpopulations were identified from side scatter (SSC) gates and the corresponding percentage of mitochondria and mean fluorescence of the gated populations for NAO and TMRM were statistically distinct (N = 13). The mean membrane potential and cardiolipin content ranged about 100-fold across the five subpopulations. Upon titration with uncoupler, antimycin A, the change in tetramethylrhodamine fluorescence was independent of the formation of ROS, suggesting that the most polarized subpopulations were more resistant to uncoupling than less polarized populations. Calcium- and iron/calcium-induced swelling of mitochondria was measured as changes in light scattering at 540 nm and via flow cytometry. The swelling is evident in cytometry from changes in both SSC and mean TMRM fluorescence. Interestingly, the more polarized, smaller subpopulations are also more resistant to calcium/iron-induced swelling as measured from TMRM but not in membrane potential. These results demonstrate the presence of functionally distinct populations in the kidney including subpopulations that are resistant to stressors implicated in pathological states and during aging.

815 Acute Doxorubicin (DXR)-Induced Nephrotoxicity Involves Massive Oxidative Stress and an Organized Perturbation of Mitochondria-Centric Pro- and Anti-Apoptotic Genes.

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Anticancer agent Doxorubicin has enjoyed popularity for decades because of its usefulness in the management of various forms of cancers. However, its cardio- and hepatoxic potential has put severe constraints on its clinical use. This study explored DXR’s potential to cause nephrotoxicity in vivo and whether it involves oxidative stress (OS). Another important goal was to examine whether OS in kidneys modulate expression of pro- and anti-apoptotic genes in order to maintain cellular homeostasis. In order to explore all these events, fed male SD rats (~90g) were injected a single dose of DXR (12mg/Kg, ip) and sacrificed on day-8. Changes in body weight, serum chemistry and tissue biochemistry were determined; kidney tissues were analyzed for MDA concentrations, SOD activity, and genomic injury (%DNA frag). The most important goal was to evaluate the level of expression of APAF-1, Caspase-3, Bad, Bax, Bcl-2, Bcl-XL, p53 and MDM-2 genes in order to coherently link extrinsic and intrinsic pathways of cell death. Data revealed that DXR-exposed animals showed significant nephrotoxicity (5.6-fold BUN and 2.65 fold creatinine increase), paralleled by a 4-reduction in total SOD activity (0.5 fold/CP). Moreover, it was found that the most polarized subpopulations were more resistant to uncoupling than less polarized populations. Calcium- and iron/calcium-induced swelling of mitochondria was measured as changes in light scattering at 540 nm and via flow cytometry. The swelling is evident in cytometry from changes in both SSC and mean TMRM fluorescence. Interestingly, the more polarized, smaller subpopulations are also more resistant to calcium/iron-induced swelling as measured from TMRM but not in membrane potential. These results demonstrate the presence of functionally distinct populations in the kidney including subpopulations that are resistant to stressors implicated in pathological states and during aging.
816 RENAL COLLECTING DUCT AND HEPATOBILIARY
TOXICITY INDUCED BY AN ANTIRETROVIRAL DRUG
CANDIDATE.

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The introduction of antiretroviral compounds with improved viral resistance profiles and novel mechanisms of action have transformed HIV-1 infection into a manageable and chronic disease. New antiretroviral treatments, therefore, need to offer improved safety profiles, as well as sustained efficacy, to be acceptable for long-term clinical use. Compound 1 (a substituted indolepyridinium) was explored as a small molecule antiretroviral candidate. However, the preclinical safety profile in 14-day in vivo preclinical toxicity studies, characterized by renal medullary lesions, hepatobiliary toxicity and high drug concentrations in tissues after repeat dosing prompted discontinuation of Compound 1 from development. Proximal tubule and glomerular damage are the most common forms of renal toxicity encountered in pharmaceutical development. In contrast the histopathological lesions (single cell death, mitosis, renal tubular regeneration) induced by Compound 1 were identified in the collecting ducts of the medulla. This uncommon toxic signature was produced by administration via the oral or intravenous route in both rat and dog. Interestingly, genomic analysis of rat kidney indicated a signature gene expression similar to that of cisplatin renal toxicity. Although the mechanism of toxicity of Compound 1 has not been conclusively identified, it is postulated that the toxic effects of this particular compound are related to the high concentrations residing in target tissues following repeat dose-administration.

817 INTENSIFIED SYSTEMIC TOXICITY BY MELAMINE IN COMBINATION WITH CYANURIC ACID IN RATS.


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In late 2008, there was a worldwide food safety issues for melamine (Mel) contamination of infant formula. It was found that pure melamine was maliciously adulterated into milk powder and children consumed the products were suffered from urolithiasis. Trace levels of Mel and cyanuric acid (Cya) can be remained in food by the use of disinfectants, pesticides and fertilizers and/or illegal adulteration into food or feed. This study was performed to compare the toxicity of Mel alone and Mel plus Cya in rats. 9 weeks old male and female F344 rats were administrated with Mel (35, 118 & 400 mg/kg bw), Cya (150 mg/kg bw), Mel+Cya (3.5 & 3.5 & 35 & 35 mg/kg bw) via gavage once/day for 4 weeks. 1% CMC (5 ml/kg bw) was used as a vehicle control. Rats treated with Mel+Cya (35 & 35 mg/kg bw) showed death or ataxia and increase of relative organ weights of kidney and adrenal glands with crystals in distal tubules of the kidneys. WBC, RBC, HGB, HCT, MPV and serum BUN and creatinine were increased but serum albumin & urinary uric acid decreased by Mel+Cya (35 & 35 mg/kg bw). Round greenish colored crystals were detected in urine sediment at Mel+Cya (3.5 & 3.5 & 35 & 35 mg/kg bw). However, all doses of Mel or Cya alone did not induce any toxicological effects excepting urinary crystals at Mel 118 & 400 mg/kg bw. In kidney toxicity tests, TIMP-1 and VEGF in serum and KIM-1, TIMP-1, β2m and cystatin C in urine were increased in male & female rats at Mel+Cya (35 & 35 & 35 & 35 mg/kg bw). These results indicate that kidney toxicity was strengthened by combined treatment of Mel with Cya. The toxic endpoint for Mel+Cya is urinary crystals, which were found even at the low dose of 3.5 & 3.5 & 35 & 35 mg/kg bw/day; at which dose no effects were found by Mel or Cya alone. The NOAEL of Mel is estimated as 35 mg/kg bw/day and that of Cya is higher than 150 mg/kg bw/day. The threshold level of urinary crystal formation is lower than each 3.5 mg/kg bw/day for Mel+Cya.

818 RESVERATROL REDUCTION OF CISPLATIN MEDIATED RENAL OXIDATIVE STRESS AND TOXICITY.

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The cancer chemotherapeutic agent cisplatin is associated with dose limiting nephrotoxicity as a serious adverse effect. The development of interventions to delay or prevent the cisplatin associated renal toxicity would be of direct clinical rel-

819 3, 4-DICHLORONITROBENZENE AND 1, 2, 3-TRICHLORO-5-NITROBENZENE Nephrotoxicity IN ISOLATED RENAL CORTICAL CELLS FROM MALE FISCHER 344 RATS.

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Chloronitrobenzene compounds have previously been shown to induce nephrotoxicity in a rat renal cortical slice model. This study uses isolated renal cortical cells (IRCC) from male Fischer 344 rats to explore two chloronitrobenzene compounds' potential to induce nephrotoxicity, including determination of whether the mechanism resulted from direct toxicity or from metabolites. Nephrotoxic potential was determined by incubating 3, 4-dichloronitrobenzene (3, 4-DCNB) or 1, 2, 3-trichloro-5-nitrobenzene (1, 2, 3-TCNNB) at concentrations of 0 - 1.0 mM with the IRCC (4 x 10^6 cells/mL) for 30 min at 37oC under an oxygen atmosphere. The tissue was next incubated for 90-120 min in 3 mL oxygenated Krebs containing 0, 75, or 150 mg/mL cisplatin in an oxygen atmosphere under the same conditions. Loss of membrane integrity was evaluated as leakage of lactate dehydrogenase (LDH) following and occurred at 120 min. Biomarkers of oxidative stress included: lipid peroxidation, glutathione peroxidase activity and expression and protein carbonyls. Lipid peroxidation was increased (p<0.05) by 75 and 150 mg/mL cisplatin relative to VEH values. A 30 min pre-incubation with RES prevented cisplatin-mediated increase in lipid peroxidation and protein carbonyls. In addition, a 30 min pre-incubation with RES followed by cisplatin exposure in the absence of RES was sufficient to reduce cisplatin cytotoxicity. These findings support the hypothesis that RES pre-incubation diminishes cisplatin renal toxicity and early changes in oxidative stress. (Supported by NIH Grant INBRE 3P20RR016477-0954).

820 CLEARANCE OF PERFLUOROOCTANOATE IN ISOLATED PERFUSED KIDNEYS FROM MALE AND FEMALE RATS – EFFECTS OF PROTEIN BINDING AND RENAL ORGANIC ANION TRANSPORT INHIBITORS.

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Rat renal clearance of perfluorooctanoate (PFO) is sex-dependent, being more than 20 times faster in females than in males. In rats, PFO is metabolically inert, primarily binds to serum albumin in the blood, and is actively secreted and reabsorbed in the kidney via organic anion transport proteins. In order to better understand the renal clearance mechanism of PFO in rats, we studied PFO clearance kinetics in isolated perfused kidneys (IPK) from male and female rats under a range of bovine serum albumin (BSA) concentrations. At 6% BSA, PFO renal clearance (CLR) values were comparable to in vivo CLR values indicating that the IPK model is a useful tool to study the renal clearance of PFO. Unbound PFO fractions (fu) were determined to be 3.8, 5.9, 9.8, and 26.8% in perfusates at 6, 2, 4 and 0.25% of BSA, respectively. In general, proportionally higher CLR values for male rat kidneys were observed at lower BSA concentrations. However, for female rat kidneys, higher PFO unbound fractions at lower BSA concentrations did not result in expected higher CLR. We are currently working on IPK experiments in the presence of inhibitors specific to organic anion transporters (Oats) and organic anion transporting polypeptides (Oaptpl1). Our results will shed light on the role of protein binding, tubular basolateral secretion and apical reabsorption of PFO renal clearance in rats.
Kidney injury molecule-2 (Kim-2) or T cell. Ig domain, mucin domain (Tim-2) belongs to the receptor family of cell surface molecules expressed on kidney, liver and T cells. Previous studies have revealed that Kim-2-deficient mice (Kim-2−/−) are more susceptible to Th2 mediated immune response. Here we investigated the phenotypic response of Kim-2−/− mice to cisplatin-induced inflammation, apoptosis and mitochondrial damage in the kidney. Male Balb C wild type (Kim-2+/+) and knockout (Kim-2−/−) mice (25-29 g) were administered with 20 mg/kg cisplatin ip and were sacrificed at 0, 24, 48 and 72 hours (n=4/group/time point). A lethality study (n=10) suggested that a 20 % lethal dose of cisplatin (20 mg/kg) in wild type mice resulted in 70 % mortality of Kim-2−/− mice after 72 h. The Kim-2−/− mice showed ~2-fold higher injury as estimated by blood urea nitrogen and serum creatinine at 48 h that further escalated at 72 h as compared to Kim-2+/+. Histologically, the outer stripe of outer medulla showed more pronounced apoptosis and necrosis in the proximal tubules in the Kim-2−/− as compared to Kim-2+/+. A significant increase in the number of apoptotic cells (TUNEL staining) was observed by 48 h overall incidence of CIN is roughly 3%, it may approach 50% in patients with kidney tubular cell proliferation (Ki67 staining) in both groups decreased at 24 h and peaked at 72 h with no difference amongst the two groups. Further analysis of genes involved in inflammation and apoptosis revealed early expression of interleukin 1 β (IL1β) by 48 h and ~5-fold upregulation of transforming growth factor β (TGFβ) by 72 h in the Kim-2−/− mice as compared to wild type. In conclusion the increased expression of the proinflammatory and proapoptotic genes IL1β, TGFβ, higher number of apoptotic cells and pronounced increase in injury and mortality of the Kim-2 deficient mice suggests a protective role of Kim-2 in cisplatin induced nephrotoxicity.

Comparative nephrotoxicity of low-osmolar and iso-osmolar iodinated contrast agents.

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Contrast-induced nephropathy (CIN) is the third leading cause of hospital-acquired renal failure, responsible for 11% of cases. CIN is associated with prolonged hospitalization and the mortality rate of CIN may be as high as 6-35%. While many of the toxic effects of this metal and can be used as a model for long-term chronic studies. In this study, this cell culture system was utilized to study global gene expression differences during the commonly employed short term exposure (24 h) and a 13 day long-term exposure. Three doses of cadmium ranging from non-toxic to sub-lethal concentrations for the 24 h (9, 27, and 45 μM) and the 13 day long-term exposure (4.5, 9, and 27 μM) were used to model acute and chronic cadmium exposure respectively. Total RNA was purified from exposed cultures and subjected to global gene expression analysis using the Affymetrix 133 Plus 2.0 array. There were a total of 1,421 differentially expressed genes specific to acute exposure, 536 specific to chronic exposure, and 387 differentially expressed genes common to both exposure conditions. Acute cadmium exposure elicited many genes in the stress response such as the heat shock group, proliferative genes, and many genes associated with cell cycle, apoptosis, and cell cycle check points. The majority of these genes did not remain differentially expressed at the later 13 day exposure time. Many of the differentially expressed genes specific to chronic cadmium exposure were found to be members of a wide range of developmental pathways. The results of these studies show a fundamental difference in the global gene expression pattern despite the fact that there was sub-lethal toxicity during both exposure conditions.

Regulation of bisphenol A-induced apoptosis in human embryonic kidney cells.

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Bisphenol A (BPA) is an environmental contaminant and putative endocrine disruptor. Although emerging evidence indicates that exposure to BPA modulates human endocrine responses, little is understood about the mechanisms underlying these effects. Our laboratory has been studying cellular responses to BPA. Using human embryonic HEK293 kidney cells we found that BPA (1-30 microM) induces apoptosis. Expression of transforming growth factor (TGFβ) beta, an inhibitor of kidney cell apoptosis, was also diminished. Previous reports indicate that peroxisome proliferator-activated receptors (PPAR) regulate cellular responses including apoptosis and glucose metabolism in individuals with metabolic disorders such as progressive kidney disease. BPA treatment increased expression of PPARβ/δ mRNA three-fold; mRNA levels remained elevated for 48 h. Treatment of PPARs requires intracellular calcium. To examine potential crosstalk between calcium signaling and PPAR expression following BPA exposure, we established a stable human embryonic kidney cell line over expressing a ligand-sensitive calcium-permeable channel (HEK/TRPV1). Both wild type and HEK/TRPV1 cells
exhibited DNA fragmentation following BPA treatment; cells over expressing the TRPV1 receptor exhibited increased levels of apoptosis. Activating the TRPV1 receptor with capsaicin (5 microM) further enhanced apoptosis. In addition, BPA-induced inhibition of TGFBeta expression was observed in wild type, but not HEK/TRPV1 cells. Levels of downstream mediators including the pro-inflammatory cytokine TNF-alpha following BPA administration in both cell types. Taken together, our results indicate that calcium and TGF beta signaling may be inversely regulated in BPA-induced responses. We speculate that these changes mediate, in part, the renal toxicity of BPA.

Many inhabitants who drink water with high concentrations of fluoride (F) are suffering from endemic fluorosis. To clarify the effects of F on individuals with impaired kidney function, F was administrated to ICR-derived glomerulonephritis (ICGN) mice at 0, 25, 50, 100, and 150 ppm in drinking water for 4 wk. F was also administered to ICR mice with normal kidney function at 0 and 150 ppm in the drinking water. Blood was sampled from the tail artery of each mouse twice a week and the blood urea nitrogen (BUN) in the serum was determined. The creatinine (CRE) or F concentrations in serum was also determined under the same protocol. The mean values on the body weight, relative organ weights, BUN, and CRE on the last day of the observation period was calculated. When a mouse was died during the observation period, the date from the day closest to the death was assigned. All ICGN mice in the 150-ppm group were died during the observation period. In contrast, no ICR mice were died even in the 150-ppm group. Mean body weight value of the ICGN mouse exposed to 150 ppm was significantly lower than those in the other groups. Mean value of BUN in the 150-ppm administered ICGN mice was significantly higher than those in the other groups. The mean CRE in the 150-ppm ICGN mice was also significantly higher than those in other groups. The mean relative liver weight of the 150-ppm group was significantly higher than those in other groups. Kidney insufficiency enhances F toxicity and, in turn, F deteriorates renal function even more.

DNA fragmentation is considered an endpoint of cell death pathways occurs both before and after the point-of-no-return. It is produced by nine cytotoxic (apoptotic) DNA endonucleases, which, despite some differences in molecular and enzymatic characteristics, catalyze the same reaction of DNA destruction. The relationships between the endonucleases are unknown. Our new findings indicate that at least one apoptotic endonuclease, DNase I, regulates the expression of another apoptotic endonuclease, EndoG, in various in vitro and in vivo models of toxic kidney tubular epithelial cell injury. We tested three models of kidney injury using DNase I knockout and wild-type mice: (1) ischemia-reperfusion by clamping of renal pedicles for 50 min followed by reflow for up to 96 hours; (2) cisplatin (20 mg/kg) nephrotoxicity, and (3) rhodobromolysis kidney injury induced by intramuscular glycerol injection (50% glycerol, 8 ml/kg). In all these models, in wild-type mice, EndoG was induced and translocated to nuclei, inducing DNA fragmentation and cell death by both apoptosis and necrosis. At the same time, the EndoG induction was significantly restricted in DNase I knockout mice, which were protected against the injuries. Two other apoptotic endonucleases, DNase II and CAD, remained unaffected by DNase I inactivation. Overexpression of DNase I-CFP fusion protein in cultured mouse tubular epithelial TKPTS cells was cytotoxic and caused the induction of EndoG after treatment with cisplatin or hemin (in vitro model of rhodobromolysis), while overexpression of CFP alone did not result in any induction of EndoG. Overexpression of EndoG-CFP in TKPTS cells was also cytotoxic but did not cause induction of DNase I. These observations indicate that there is a hierarchy among cytotoxic endonucleases: DNase I serves as an upstream regulator of EndoG, and suggest a possibility that endonucleases, similar to other apoptotic enzymes, may act as a network.

Many inhabitants who drink water with high concentrations of fluoride (F) are suffering from endemic fluorosis. To clarify the effects of F on individuals with impaired kidney function, F was administrated to ICR-derived glomerulonephritis (ICGN) mice at 0, 25, 50, 100, and 150 ppm in drinking water for 4 wk. F was also administered to ICR mice with normal kidney function at 0 and 150 ppm in the drinking water. Blood was sampled from the tail artery of each mouse twice a week and the blood urea nitrogen (BUN) in the serum was determined. The creatinine (CRE) or F concentrations in serum was also determined under the same protocol. The mean values on the body weight, relative organ weights, BUN, and CRE on the last day of the observation period was calculated. When a mouse was died during the observation period, the date from the day closest to the death was assigned. All ICGN mice in the 150-ppm group were died during the observation period. In contrast, no ICR mice were died even in the 150-ppm group. Mean body weight value of the ICGN mouse exposed to 150 ppm was significantly lower than those in the other groups. Mean value of BUN in the 150-ppm administered ICGN mice was significantly higher than those in the other groups. The mean CRE in the 150-ppm ICGN mice was also significantly higher than those in other groups. The mean relative liver weight of the 150-ppm group was significantly lower compared with the control group. There were no significant differences in there indexes between the 0 and 150 ppm for ICR mice. The serum F in ICGN mice exposed to 100 ppm that remained alive was significantly higher than those in other groups. There were no significant differences in there groups. Kidney insufficiency enhances F toxicity and, in turn, F deteriorates renal function even more.
commercial applications, and have been found to be both ubiquitous and highly persistent in the environment. Previous studies have indicated robust developmental toxicity associated with exposure to PFOS, PFOA, and PFNA individually in laboratory rodent models. However, multiple PFAs are present in the environment and detectable to varying extent in humans. Hence, effects of these chemicals in mixtures must be taken into consideration for their health risk assessment. The current study examines the developmental effects of various mixtures of PFAs and makes comparisons to exposures to individual compounds. Timed-pregnant CD-1 mice were given either alone (8 mg PFOS/kg, 4 mg PFNA/kg) or in mixtures (4 mg PFOA/kg + 2 mg PFNA/kg; 4 mg PFOA/kg + 6 mg PFOS/kg or 6 mg PFOA/kg + 2 mg PFNA/kg) by oral gavage daily from gestation day 1-17; controls received 0.5 % Tween vehicle. PFOS, PFOA and PFNA singly produced developmental effects as previously reported. In mixtures, PFAs appeared to have a dose additive effect on maternal weight gain, pup body weight, as well as maternal and neonatal liver weights. In contrast, PFAAs in mixtures induced a less-than- dose additive effect on neonatal mortality. In particular, the PFOS + PFOA group responded less than PFOS or PFOA alone, where as PFOS + PFNA had no response at all. These data suggest that prenatal exposure to a mixture scheme of PFAAs with higher carbon chain length (C-8 and C-9) containing either a carboxylic or sulfonic functional group produce additive effects on some endpoints and less-than-additive effects on neonatal mortality in CD-1 mice. This abstract does not necessarily reflect U.S. EPA policy.

MAMMARY GLAND DEVELOPMENT AND RESPONSE TO PRENATAL ATRAZINE EXPOSURE IN THE SPRAGUE-DAWLEY AND LONG-EVANS RAT.


Mammary gland (MG) tumor development in Sprague-Dawley (SD) rats is increased by long-term dietary exposure to the chlorotriazine herbicide atrazine (ATR). ATR is proposed to cause these changes in the adult SD rat by altering hormonally-regulated estrous cyclicity. In Long-Evans (LE) rats, both puberty and MG development are delayed by brief prenatal ATR exposure. Prenatal exposure has not been tested in SD rats. We have observed that dimethylenz[a]anthracene (D MBA) treatment results in a greater incidence of MG tumors in LE compared to SD rats and we hypothesize that this increased susceptibility is due to strain differences in the rate of MG differentiation, and that these differences are compounded by exposure to ATR. The purpose of this study was to assess the differences in MG development of female offspring of vehicle and ATR-exposed LE and SD rats. Timed-pregnant LE and SD rats (n=9/treatment group) were orally gavaged with 0, 12.5, 25, or 50 mg ATR/kg body weight 2x/day on gestational days 15-19. Mammary glands were collected from female offspring on PNDs 4, 25, 33, and 45 and MG whole mounts were scored according to previously established developmental criteria. MG development was delayed by ATR on PND4 and PND25 in the SD rat at 50 mg/kg, and on PND33 in both strains at 25 and 50 mg/kg (p< 0.05). At PND45, MG differentiation was delayed in both strains at all doses (5 mg/kg, p< 0.01 in LE, p< 0.05 in SD; 25 mg/kg, p< 0.01 in LE, p< 0.001 in SD; 50 mg/kg, p< 0.001 in LE and SD). Additionally, MG of SD offspring were more much developed than that of LE offspring at respective ages, although age at VO was not different between strains. In summary, ATR delayed MG development in both strains, and the VO of SD was 25.5 mg/kg/day (12.5 mg/kg/dose), despite the difference in MG maturity between the strains. These data suggest less developed MG in the LE may render this strain more susceptible to DMBA-induced tumor promotion. This abstract does not necessarily reflect EPA policy.

DEVELOPMENTAL TOXICITY EVALUATION IN THE CYNOOMOLUS MONKEY: VARIABILITY OF PREGNANCY LOSS AND STATISTICAL GROUP SIZE CONSIDERATIONS.

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The cynomologus monkey (Macaca fascicularis) provides the established primate model for preclinical assessment of developmental toxicity. Group sizes are generally smaller than those used in rodent and rabbit studies. We explored in the monkey model the pregnancy outcome in control animals from 78 embryofetal development (EFD) studies terminated either on gestational day 100 or 150 and 14 pre-/postnatal development (PPND) studies until day 7 postpartum, comprising 1069 pregnancies between 1981-2000. Neither study type nor route/duration of dosing affected pregnancy outcome. Hazard rate was higher pre-1989 (0.3, 104/347) compared to post-1989 (0.13, 94/722). Hazard was greatest in early gestation (< gestational day 50) and at parturition. Monte-Carlo simulation experiments resulted closely correlated to the actual losses indicating that losses within and between studies were independent. Reference data for the variability and likelihood of pregnancy success could be derived. Systematic data were generated for predicting pregnancy outcome and hazard detection relative to group size at study initiation. For example, EFD studies with an initial vehicle group size of 16 and 20 will have 13 and 16 ongoing pregnancies, respectively, at gestational day 100 with 80% probability. For PPND studies with initial vehicle group sizes of 16, 20 and 28, life infant number at day 7 postpartum will be 9, 11 and 16, respectively, with 80% likelihood. With regard to statistical power, a PPND study initiated with a group size of 20 could detect a trebling of the hazard to live infant outcome. Moreover, the simulation data provide an objective tool facilitating decisions in ongoing studies whether supplementation with additional pregnant animals is indicated or not.

NEUROPATHY TARGET ESTERASE (NTE) MIGHT BE PLAYING A RELEVANT ROLE DURING CELL DIFFERENTIATION.


Neuropathy Target Esterase (NTE) is a protein known because is the target of a delayed polynepathy caused by exposure to certain organophosphorus compounds. However, certain in vivo studies suggest that NTE might be relevant during the embryonic development because mice deficient in both NTE alleles are not viable. We have discriminated, using organophosphorus inhibitors, NTE activity among the pool of carbohydrate esterases found in embryonic stem cells (ESC) from mouse belonging to D3 line. These cells express an NTE activity of 23 nmol phenol/min/mg protein (0.99 mg phenol/min/106 cells). The expression of Pnpa 6 (the gen codifying for NTE) increased in D3 ESC immediately after initiated the differentiation, reaching a maximum of expression around 5 or 30 hours after triggered the differentiation when cells were cultured in monolayer or forming embryonic bodies, respectively. This maximum of Pnpa6 expression also correlated with a maximum of enzymatic activity in monolayer cultures. The NTE activity and the Pnpa6 expression returned to basal levels after 48 hours (in monolayer cultures) and 10 days (in EBS) of differentiation, respectively. The changes in the Pnpa6 expression in D3 embryonic bodies did not correlate with changes noted in the expression of intermediate neurofilament (gene marker of neuroectoderm development), alpha-fetoprotein and Amnionless (both gene markers of endoderm development) and myosin heavy chain and Flk1 (both gene markers for mesoderm development). These results suggest that NTE may play an important role in the initial stages of cell differentiation and that mouse ESC might be a model for studying this role. Acknowledgment: This study was supported by Ministry of the Environment of the Government of Spain (Grant A051/2007/3-1-4).

SPECIES DIFFERENCES IN CATALASE AND ALCOHOL DEHYDROGENASE ACTIVITY IN MICE AND RABBITS.

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Predictions of human risk for methanol (MeOH) developmental toxicity are based largely on rodent studies. Unlike humans, rodents use catalase rather than alcohol dehydrogenase (ADH) to metabolize MeOH, and use catalase and a non-saturable folate pathway for metabolizing the toxic formic acid (FA) metabolite, thereby preventing its accumulation and the subsequent acute toxicity observed in humans. However, catalase in both rodents and humans also detoxifies reactive oxygen species (ROS), which are implicated in the developmental toxicity of MeOH in rodents. Due to this potentially confounding dual role of catalase for MeOH in rodents but not humans, rodents may not constitute a reliable animal model for accurately assessing the human potential for MeOH teratogenesis. We previously showed that New Zealand white (NZW) rabbits more closely than mice reflect primary MeOH and FA disposition, exemplified by slower MeOH clearance and greater FA accumulation. Herein we report preliminary data for the enzymes responsible for MeOH and FA metabolism in rabbits. Catalase activity in hepatic tissue from male NZW rabbits and CD-1 mice was assessed using the Ferrous Oxidation in Xylenol Orange (FOX) assay. The mean catalase activity in mice was about 3-fold higher than that in rabbits (p < 0.0001), indicating a substantial species difference. Compared to mice, the hepatic catalase activity of rabbits more closely approximated that reported in primates, providing biochemical evidence that rabbits may be a more appropriate animal model for assessing the potential teratological risk of MeOH in humans. Studies examining the activity of ADH and other relevant enzymes in adult and fetal mice and rabbits are ongoing, the results of which will permit a more comprehensive assessment of these models. (Support: Methanol Fdn., CFFR).
The nanotechnology (NT) industry is projected to expand to $34.5 billion in 2012 and exceed $1 trillion by 2015. Despite extensive interest by regulatory agencies and the fact that 69 million women make up the U.S. workforce (about 5 million are in manufacturing sector) with about 75% being of reproductive age, nothing is currently known about the impact of nanomaterials (NM) on female reproductive/foetal health. A study was thus carried out with pregnant B6C3F1 mice to determine whether inhaled cadmium oxide (CdO) NP could pose a risk to the developing fetus. Dams, exposed once for 5 h (by nose-only inhalation) to an airborne CdO (about 30.3 μm) concentration of 550 μg Cd/m3, were sacrificed 14 h after exposure and their lungs, blood, uterus, and placentas collected and weighed; numbers of viable and resorbed fetuses were also determined. Biological tissues, analyzed for Cd by atomic absorption, revealed that pulmonary and blood Cd levels in exposed dams averaged 5 μg Cd/g tissue and 120 ng Cd/ml compared to non-detectable levels in controls. Uterus and placental burdens of Cd were 14 and 27 μg Cd/g tissue collected at the same early time point post-exposure; placental levels of Cd in the air-exposed mice were below detectable limits. The placental Cd level appeared elevated, a placental transfer pathway involving endocytic uptake by the visceral placentation accumulation. Inhalation of Cd NP more than doubled the incidence of dams demonstrating resorbed fetuses and in utero litter size decreased by 36% compared to the air counterparts. Results show that Cd from inhaled CdO NP can reach the placenta and produce fetal toxicity. NYU NIEHS Center Pilot Project and NIEHS ES017427-01.

**836 COMPARISON OF RAT AND RABBIT CONCEPTUS RESPONSES TO INHIBITORS OF HISTIOTROPHIC NUTRITION.**

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Regulatory agencies often require developmental toxicity testing in both rodent (usually rats) and non-rodent species (usually rabbits). Discordant responses between these species are common, and in such cases, the most sensitive species is assessed most relevant to humans. Understanding the mechanisms behind species discordance would enhance the scientific basis for animal-human extrapolations and could reduce the need for testing in two species. One mechanism of developmental toxicity thought to be unique to rodents is inhibition of histiotrophic nutrition (HN), a placental transfer pathway involving endocytic uptake by the visceral yolk sac, degradation by lysosomal cysteine proteases, and delivery to the embryo via the vitelline circulation. HN is considered not relevant to humans due to the lack of a visceral yolk sac, and some have proposed that it is also not operative in rabbits. To determine if the HN pathway is necessary for rabbit development, mid-somite stage New Zealand White rabbit and CdO CRB rat conceptuses were exposed in whole embryo culture to inhibitors of endocytosis (cubilin A, 0.05-10 μg/ml), lysosomal cysteine proteases (leupeptin, 0.25-10 μg/ml) or both (trypsin blue, 500-2500 μg/ml). After 48 h, effects on embryo viability, growth, and morphology were compared between the two species. All three HN inhibitors adversely affected multiple end points of development in both species. Rat and rabbit embryos were equally sensitive to trypsin blue and cubilin A as indicated by their identical no-effect concentrations (NOEC) for each inhibitor (trypsin blue: 500 μg/ml; cubilin A: 1 μg/ml), although some specific end points exhibited differential sensitivity. For leupeptin, the rabbit NOEC was 2.5 μg/ml while the rat NOEC was considered <2.5 μg/ml due to effects at all concentrations tested. These results indicate that both species require the HN pathway, which contrasts with earlier reports that rabbits are insensitive to HN inhibition.

**837 ALTERED GENE EXPRESSION IN THE BRAIN FOLLOWING REPEATED CHLORPYRIFOS EXPOSURE IN LATE PREWEANING RATS.**

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The organophosphate insecticide Chlorpyrifos (CPS) was removed from home use in 2001. However, it is still used widely in agricultural settings, posing an exposure risk to agricultural workers, their children, and proximal communities. Environmental drift and take-home exposure pathways put children in agricultural communities at higher risk than adults to CPS exposure and to any potential detrimental effects on brain development. Previously, we measured acetylcholinesterase (AChE) following daily CPS exposure (5 mg/kg) from PND10 through PND16 using three of the most common routes of administration, orally in oil, subcutaneously in oil, and subcutaneously in DMSO. Peak inhibition of brain AChE on PND16 occurred between 4-6 h post-treatment with the greatest inhibition in the group exposed to CPS subcutaneously in DMSO. In the present study, we examined the induction of the expression of several genes in the hippocampus and medulla. The hippocampus and medulla were both isolated by laser capture microcopy (LCM). RNA was isolated and gene expression was determined via qRT-PCR. The expression of the immediate early gene c-Fos was increased in all groups indicating cellular excitation. The expression of brain derived neurotrophic factor (BDNF) and N-methyl-D-aspartic acid receptor 1 (NMDAR1), were also increased (1 to 4 fold) in response to exposure to CPS. The expression of superoxide dismutases 1, 2, and 3 were all increased following exposure. These increases were observed in both hippocampus and medulla suggesting an increased production of ROS in response to exposure. It has been suggested that CPS exposure results in the induction of oxidative stress in addition to acetylcholinesterase (AChE) inhibition. Both AChE inhibition and oxidative stress have been shown to alter neural cell migration and differentiation, causing neural developmental disorders such as ADHD.

**838 ARSENIC DELAYS THE DIFFERENTIATION OF MOUSE MUSCLE CELLS THROUGH THE REPRESSION OF MYOGENIN AND ALTERATION OF DNA METHYLATION.**

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Arsenic is a toxicant commonly found in water systems around the world. Evidence from epidemiological studies indicates that chronic arsenic exposure can result in cancer, central nervous system and sensory deficits, effects on development, and neuromuscular deficits. However, the molecular mechanism of arsenic's toxicity remains largely unclear. We are using the C2C12 mouse myoblast cell line to investigate the effects of sodium arsenite on differentiation. Myotube formation was delayed upon exposure to 20μM arsenic. Additionally, mRNA levels of myogenin, a transcription factor that plays an essential role in skeletal muscle differentiation, was significantly reduced in arsenic treated cells. To investigate the mechanisms behind the reduction in myogenin expression, changes in methylation patterns in the promoter region and the exon 1 of myogenin (-473 to +200) were examined by methylation-specific PCR and bisulﬁte genomic sequencing. Sequence ampliﬁed by primer pairs contained 19 CpG sites. Hypermethylated CpGs were found at CpG No. 3 and 8 (-236 and -126, respectively), whereas hypomethylated CpGs were found at CpG No. 7 (-207) in arsenic exposed cells. Arsenic exposure delays myotube differentiation and represses myogenin expression in part by altered DNA methylation in the myogenin promoter.

**839 DEVELOPMENTAL EXPOSURE OF CD-1 MICE TO PFOA IDENTIFIES THE MAMMARY GLAND AS A LOW DOSE TARGET TISSUE.**

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The surfactant perfluorooctanoic acid (PFOA) has become an environmental contaminant due to its widespread industrial applications. Previous studies by our lab indicated that exposure to 5 mg/kg/d PFOA during gestation delayed mammary gland development in exposed female offspring. To investigate the effects of low-dose PFOA exposure on the mammary gland, timed-pregnant CD-1 mice were orally dosed with 0.0, 0.01, 0.1, or 1.0 mg PFOA/kg body weight on gestation days (GD) 10-17. Litters and pregnant dams were removed from pups at necropsy on postnatal days (PND) 1, 4, 7, 14, and 21. When compared to controls, all exposed litters displayed aberrant mammary gland development. These effects were most prominent on PND 21 when all treatment groups were significantly different compared to controls for mammary gland score (p < 0.002). At PND 21, the pups in the highest exposure group (1.0 mg/kg) displayed the most impaired mammary gland growth, with low mammary gland scores, low longitudinal and lateral epithelial growth, fewer visible branch points, and fewer visible terminal end buds (p < 0.05). Although there was no effect on body weight due to treatment at any age evaluated, there was a difference in liver/body weight ratios in the 1.0 mg/kg dose group compared to controls evident from PND 1 to 14 (p = 0.002), which was likely due to significantly greater liver weights at PND 4, 7, and 14 in the 1.0 mg/kg exposed pups. These data suggest that the lowest observable effect level of late gestational exposure to PFOA on liver/body weight ratio is 1.0 mg/kg, but is 0.01 mg/kg for mammary growth and development.
developmental effects. The residual tissues from this study are currently being analyzed to determine the mechanistic pathways of low-dose PFOA toxicity in the mammary gland. This abstract does not necessarily reflect NIEHS policy.

**840** ANALYTICAL METHOD VALIDATION FOR DIISOBUTYL PHthalate in rodent FEED in SUPPORT of DEVELOPMENTAL and REPRODUCTIVE TOXICOLOGY STUDIES.


Diisobutyl phthalate (DIBP), a phthalate acid ester commonly used as plasticizer, in mixtures with nonylphenols, and as a fuel oil marker, has long been suspected to be both a developmental toxin and reproductive toxicin as well as a possible carcinogen in rodents. Due to potential environmental exposure of phthalate esters and their continued use in consumer products in general, renewed concerns have been raised regarding their toxicological effects. An analytical method using UPLC was successfully developed and validated to accurately quantify DIBP from ~6 ppm to ~10,000 ppm (mg/kg) in NIH-07 and NTP-2000 rodent feed, in support of future NTP studies to evaluate developmental and reproductive toxicity in rodents. The UPLC methodology has resulted in better resolution, higher sensitivity, and faster analysis time as compared to regular LC methods reported in the literature. The analytical method employed a liquid extraction approach with an experimental limit of quantitation (ELOQ) of 5 ppm in both feeds. Linearity (r > 0.999; weighted 1/x2 linear regression) was demonstrated up to 800 ppm for NIH-07 and 500 ppm of quantitation (ELOQ) of 5 ppm in both feeds. Linearity (r > 0.999; weighted 1/x2 linear regression) was demonstrated up to 800 ppm for NIH-07 and 500 ppm for NTP-2000 feed. Limits of detection (LOD) were 0.7 ppm and 0.3 ppm, for NIH-07 and NTP-2000, respectively. Method verification experiments utilizing a dry dilution scheme were successfully conducted at a test concentration of 12,000 ppm in both feeds. Percent relative error versus nominal concentrations was ≤5% for all formulations for both feed types. Precision was demonstrated with %RSD values ≤3% across both feed types. Extraction efficiency for DIBP, expressed as percent recovery, was 84% for NIH-07 and 81% for NTP-2000. DIBP was demonstrated to be stable in both feed vehicles for up to 42 days and for up to 7 days in the presence of rodent excreta. These results suggest this is a viable method for analysis of DIBP in rodent chow in support of toxicology studies.

**841** DEVELOPMENTAL IMMUNOTOXICITY OF METHYLMERCURY: THE RELATIVE SENSITIVITY of DEVELOPMENTAL AND IMMUNE PARAMETERS.

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In recent years there has been an increasing focus on risk assessment in children as a specifically susceptible population. However, current developmental and reproductive toxicity protocols include only a limited set of parameters for effects on the developing immune system. In this study a wide range of immunological parameters were included in a pre- and postnatal developmental toxicity study. Dose-response data were compared to determine the relative susceptibility of different immune and developmental parameters. Mated female Wistar rats were dosed daily by gavage with methylmercury (0, 0.4, 0.6, 1.0, 1.5, 2.0 mg/kg bw/day) from gestation day 6 to postnatal day (PND) 10. In addition to general, reproductive and developmental parameters, a wide range of immunological parameters were assessed in male pups at PND 21, 42 and 70. The T cell-dependent antibody response to Keyhole Limpet Hemocyanin (KLH) was assessed following subcutaneous immunizations on postnatal days 21 and 35. Dose-response data were analyzed using the Benchmark dose (BMD) approach by fitting dose-response models to the various endpoints. BMDs were determined using pre-determined benchmark responses. Methylmercury induced effects on developmental parameters such as growth parameters and pup mortality. Effects found on the immune system differed at the three time points and consisted mainly of effects on functional parameters. The parameter with the lowest BMD was the primary KLH-specific IgG antibody response which showed a dose-dependent decrease with a BMD of 0.12 mg/kg bw/day (CI 0.05-0.38). These data show the relative susceptibility of the developing immune system and thereby illustrate the relevance of immune parameters in reproductive and developmental protocols.

**842** EFFECTS OF FIGITUMUMAB, AN ANTI-IGF1R MONOCLONAL ANTIBODY, ON EMBRYO-FETAL DEVELOPMENT in CYMONOLGUS MONKEYS.

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Insulin-like growth factor (IGF) signaling has been linked to tumor cell survival and tumorigenesis. This has led to development of an anti-IGF1 receptor monoclonal antibody (figitumumab) as an anti-cancer therapeutic. As part of the required safety evaluation, an embryo-fetal developmental toxicity study was conducted in the cynomolgus monkey. Figitumumab was administered once weekly by intravenous dosing at 3 dose levels during the period of major organogenesis (gestation days 20 to 48) with scheduled cesarean section around gestation day 100. Maternal endpoints included clinical observations, food consumption, body weights, hematology, toxicokinetics and immunogenicity. Fetal evaluations included viability, body weights, external, visceral and skeletal examination (and measurements), drug exposure and immunogenicity. There was a robust, dose-dependent increase in embryo-fetal loss in all treatment groups in the presence of decreased food consumption and slight body weight loss. Treatment-related embryofetal developmental toxicity was observed as growth retardation/reduced fetal body weights in all treatment groups with corresponding developmental delays in morphology. Treatment-related fetal structural malformations were also observed in the mid and high dose groups. Although most of these effects may be attributable to inhibition of IGF signaling in the mother and/or placenta (specifically the pregnancy loss and fetal growth effects), the mechanism of the fetal morphology changes is difficult to determine based on literature data suggesting that little or no immunoglobulin crosses the placental barrier during the first trimester in cynomolgus monkeys due to an apparent lack of the necessary active transport system at this developmental stage. This study demonstrates a case where first trimester-only dosing of monoclonal antibodies in the non-human primate was able to identify a hazard to developmental toxicity.

**843** FETAL EXPOSURE TO BREVETOXIN IN CD-1 MICE: DOSE TO TISSUE and EFFECTS ON LYMPHOID POPULATIONS.

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Brevetoxins are produced by Karenia brevis, the algae causing Florida Red Tide. Previously, brevetoxin-3 was detected in fetuses of dams exposed during gestational days (GD) 6 – 19. No developmental toxicity was associated with these exposures (The Toxcologist 108 [1]). The purpose of this study was to quantitate brevetoxin in fetal tissue following maternal exposure. Two pregnant CD-1 mice administ- ered 0.67 μg3H-brevetoxin 3/day, beginning on GD 6. Dams were euthanized on GD 19. Pup brains, kidneys, lungs, livers and spleens were pooled from each litter for processing an analysis. Concentration of brevetoxin equivalents present in mater- nal tissues ranged from 0.25 ng/g (spleen) to 0.44 ng/g (kidneys and GI tract). Smaller, but quantifiable amounts of brevetoxin were measured in fetal liver (0.09 ± 0.03 ng/g), and brain (0.09 ± 0.04 ng/g), and brain 0.10 ± 0.04 ng/g). Because breve- toxin was found in liver, the source of fetal white blood cells, further studies were conducted to examine T and B lymphocyte populations in maternal and fetal tis- sues. Dams were exposed to brevetoxin-3 as described above. Thymus glands, spleens and livers were harvested, lymphocytes isolated, and analyzed by flow cy- tometry. While the percentages of CD3+ cells in liver of control and brevetoxin-ex- posed fetuses were similar, the percentage of CD19+ cells in brevetoxin exposed fetal liver was significantly greater (50% greater) than measured in control pup liver. To date, no brevetoxin-specific effects on the percentages of CD3+, CD19+, CD3+CD4+, CD3+CD8+ or CD4+CD8+ cells in fetal spleen and thymus have been found. In conclusion, we have determined that brevetoxin or its metabolites distribute to fetal liver, lung and brain, and that there may be some effect on B lymphocyte populations in liver. The potential health effects resulting from these exposures remain to be determined. Research conducted under NIEHS P01 ES10594 and FWC/FWRI Red Tide Control and Mitigation Grant 68A-2.015.
REGULATION OF VENTRAL EPITHELIAL BUD PATTERNS IN MOUSE UROGENITAL SINUS (UGS) BY ANDROGEN SIGNALING AND 2, 3, 7, 8- TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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Prostate lobes in rodents develop from buds that appear in four discrete UGS regions. Prostatic bud numbers and patterns can be altered, in different ways, by chemicals such as bisphenol A and TCDD. We investigated (a) the effects of androgens on bud patterns in the ventral UGS, and (b) if androgen signaling affects cell death by paternizing by TCDD. Pregnant mice were given mice were given 5α-di-hydrotestosterone (DHT), flutamide (antiandrogen), or placebo pellets on E13.5; and TCDD was orally given to 20% of the dams. Prostatic bud numbers and patterns can be altered, in different ways, by chemicals such as bisphenol A and TCDD. We investigated (a) the effects of androgens. Prostatic bud numbers and patterns can be altered, in different ways, by chemicals such as bisphenol A and TCDD. We investigated (a) the effects of androgens. Prostatic bud numbers and patterns can be altered, in different ways, by chemicals such as bisphenol A and TCDD. We investigated (a) the effects of androgens. Prostatic bud numbers and patterns can be altered, in different ways, by chemicals such as bisphenol A and TCDD. We investigated (a) the effects of androgens

THE EFFECTS OF L-CARNITINE ON KETAMINE-INDUCED NEUROTOXICITY IN RAT FOREBRAIN CULTURES.

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Ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is used as a general pediatric anesthetic. It is known that anesthetic drugs may cause neurodegeneration if administered during critical periods of development. L-carnitine plays an important role in attenuating brain injury associated with mitochondrial neurodegenerative disorders. The purpose of this study was to determine the dose and temporal response of ketamine, and also to investigate whether co-administration of L-carnitine could protect against or attenuate ketamine-induced cell death in newborn rat forebrain cultures. Ketamine was used as a biomarker for oxidative DNA damage. An 8-oxo-dG ELISA and antibodies to human 8-oxo- deoxyguanosine (8-oxoG) were used to explore the potential regulatory DNA repair systems. The 8-oxo-dG ELISA and immunostaining data indicate that ketamine (10 and 20 μM) caused elevated levels of 8-oxo-guanine and a reduction in mitochondrial DNA metabolism. 8-oxo-dG was also shown to be increased in DNA migration as indicated by an alkaline single-cell gel electrophoresis (Comet assay). No significant release of lactate dehydrogenase (LDH) was observed. Ketamine-induced neurotoxic effects (DNA damage) were effectively blocked by L-carnitine (30 and 100 μM), suggesting its potential protective effects via prevention of apoptosis and oxidative damage and changing cellular transcriptional responses.

EFFECTS OF METHYLMERCUry PUBESCENT EXPOSURE ON THE BRAIN AND EXPoSURESYSTEM.

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Elevated mercury levels in the sediment of waterways, mainly found in the flesh of fish and other aquatic mammals. Adult chronic exposure can lead to motor and subtle cognitive deficits. Maternal exposure during pregnancy can lead to fetal neural disorganization especially in the cerebellum and the motor cortex. However, the effect of MeHg exposure during the pubescent growth period is little understood. Female rats were exposed to 0.0, 2.5 or 5.0ppm MeHg in their drinking water for 60 days throughout the pubescent growth period. They were then mated with unexposed males. Necropsies were performed on day 15 of gestation and histological analysis performed on the brains and ovaries. Neuroanatomical changes in the brains of the MeHg subjects treated were most marked in the high exposure group. Cortical layers within the motor and visual cortices were disorganized in comparison to control groups. Within the hippocampus, an increase in pyknotic nuclei was observed in the dentate gyrus, and larger neurons displayed less Nissl substance. Cerebellar changes consisted of pyknotic nuclei in the granule cell layer and displacement of granule cells across other cerebellar layers. Purkinje cell somas and apical dendrites were decreased in size; and less Nissl substance and more vacuoles were apparent. Analysis of the ovaries from the exposed animals revealed a decrease in size in the high exposure group. The degeneration of primordial follicles in the exposed animals was identified. The numbers of corpora lutea were increased and a larger number of sinusoids were observed. Luteal cells displayed high vacuolation. These ovarian changes may result in decreased fertility in future pregnancies and hormonal changes that disrupt normal reproductive cycling in the exposed animals. Research supported by NIH, RCSI (5 G12 RR003059) COE HRSA D34 HP00001.
stand breaks in an in vitro model of mutagenesis. The pKZ1 transgenic mouse model contains a DNA construct allowing for the detection of intrachromosomal recombination events. Immunocytochemistry probing for H2A.X phosphorylation was used to assess the ability of BQ to induce DNA double strand breaks in hematopoietic cells. Primary cultures of gestational day 14 fetal livers were exposed to 5, 10, 25, or 50 μM BQ and stained for recombination. A significant increase in recombination events was observed following treatment with 25 and 50 μM BQ at all time points with the 10 μM treatment displaying a significant increase over control at 24 hrs. Hematopoietic cells treated with 25 and 50 μM BQ showed an increased number of fluorescent γ-H2A.X foci at 8 and 24hrs but this increase was found to be non-significant likely due to the small sample size. These results indicate that BQ is able to induce intrachromosomal recombination in fetal hematopoietic cells possibly through the creation of DNA double strand breaks.

Support: CIHR.

849 OBSERVATIONS OF THE EFFECTS OF NONAPARTICLES ON REPRODUCTION AND DEVELOPMENT IN DROSOPHILA MELANOGASTER AND CD-1 MICE.

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The exciting surrounding the multiple uses of nanoparticles continues to increase, while information about their potential toxicity lags behind. Because of the small size of nanoparticles (<100nm), their physiochemical properties can change allowing them to cross cellular membranes and to potentially interfere with cellular processes. Silver (Ag) and titanium dioxide (TiO2) nanoparticles are becoming widely used in popular commercial products such as foods and packaging, cosmetics and medical devices. To investigate any effect on reproduction and development, these two nanoparticle types were assessed using both Drosophila melanogaster and mice as models. Male and female Drosophila were housed together and exposed to varying concentrations of either type of nanoparticle or a vehicle control in their food (0.005% w/v to 0.5% w/v). The exposure period was 14 days and during this time, males and females were allowed to reproduce while female fecundity was recorded daily. Information taken included both oviposition and overall fertility. Both Ag and TiO2 nanoparticles significantly reduced female fecundity, particularly at 0.1% and 0.5% concentrations. In mice, pregnant CD-1 dams were orally dosed with either nanoparticle (100 nm or 1 μm), their physiochemical properties can change allowing them to cross cellular membranes and to potentially interfere with cellular processes. Silver (Ag) and titanium dioxide (TiO2) nanoparticles are becoming widely used in popular commercial products such as foods and packaging, cosmetics and medical devices. To investigate any effect on reproduction and development, these two nanoparticle types were assessed using both Drosophila melanogaster and mice as models. Male and female Drosophila were housed together and exposed to varying concentrations of either type of nanoparticle or a vehicle control in their food (0.005% w/v to 0.5% w/v). The exposure period was 14 days and during this time, males and females were allowed to reproduce while female fecundity was recorded daily. Information taken included both oviposition and overall fertility. Both Ag and TiO2 nanoparticles significantly reduced female fecundity, particularly at 0.1% and 0.5% concentrations. In mice, pregnant CD-1 dams were orally dosed with either nanoparticle (10, 100 or 1000 mg/kg) or a vehicle control on gestational day (GD) 9. Fetuses were removed from dams on GD19, and were examined for both incidence of resorptions and the incidence of morphological defects. Defects were observed in mouse fetuses particularly with TiO2 nanoparticles, though to a lesser extent than in the invertebrate studies. Together, these studies shed light on the potential toxicological implications of nanoparticles and future studies will investigate the mechanisms of this toxicity.

851 EVALUATION OF THE EFFECTS OF SURGERY AND CONTINUOUS INTRAVENOUS INFUSION ON EMBRYOFETAL DEVELOPMENT IN PREGNANT SPRAGUE-DAWLEY RATS.

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Early embryonic development is susceptible to interference by a multitude of chemical, biochemical or physiological factors. Maternal stress arising from laboratory animal manipulation during early gestation may impact the well being of the developing embryo. As a part of the developing reproductive-embryofetal toxicology facility in our laboratory, the objective of this study was to assess the impact of surgical cannulation of timed-pregnant Sprague-Dawley (SD) rats on Gestation Day (GD) 0, followed by continuous intravenous infusion through GD 6 to 15, on pregnancy outcome and embryofetal development. The females were surgically cannulated with an intravenous catheter inserted into the femoral vein and advanced into the vena cava, and administered saline at a dose rate of 2.5 mL/kg/hour from GD 6 to 15. Control animals were time-mated non-catherized, and untreated. Maternal body weights (BW) and food consumption (FC) were monitored throughout the gestation period. Pups were delivered by cesarean on GD21 and an external as well as internal exam was performed, followed by processing for skeletal exam and head exam (using Wilson’s technique). Maternal BW and FC were comparable in cannulated and non-cannulated animals, though the former showing slightly lower values. Litter size and mean fetal body weights were comparable between the cannulated and non-cannulated animals and there were no external or visceral anomalies or malformations to indicate an effect of the surgical or infusion procedures. Similarly, skeletal and Wilson’s examinations did not reveal differences from controls. The above results indicate that surgical manipulation of Sprague-Dawley rats on GD0 for cannulation followed by intravenous infusion from GD6-15 does not impact pregnancy outcome and embryofetal development.

852 EMBRYOFETAL TOXICITY OF BORIC ACID: VALIDATION OF PROCEDURES AND EVALUATION METHODS, INCLUDING CONTINUOUS INTRAVENOUS INFUSION IN NEW ZEALAND WHITE RABBITS.


As a part of the developing reproductive-embryofetal toxicology facility in our laboratory, this study was conducted to (i) confirm the effects of a known embryotoxic (ii) assess the effects of surgical cannulation of does on gestation day (GD) 0 and (iii) assess the effects of continuous intravenous infusion from GD 6 to 19, on pregnancy outcome and embryofetal development in New Zealand White (NZW) rabbits. Boric Acid (BA, identified as a Class 2 embryotoxicant by European Centre for the Validation of Alternative Methods, was selected for part (i). Timed-pregnant NZW rabbits were orally administered (5 mL/kg) 0, 125, 200, and 250 mg/kg BA in water (doses selected based on literature as well as pilot study in non-pregnant adult female NZW rabbits) from GD7 to 19. For part (ii) and (iii) a separate group of animals was intravenously infused with saline via a surgically implanted catheter from GD7 to 19 (3 mg/mL/hr; animals surgically cannulated on GD0). Maternal body weights and food consumption were monitored throughout the gestation period. Pups were delivered by cesarean on GD29 and an external as well as internal exam was performed. The fetuses were processed for skeletal (Alizarin Red S staining) and head examination (Wilson’s technique). BA treatment resulted in vaginal bleeding at 200 and 250 mg/kg and severe developmental toxicity with complete loss of surviving fetuses at 250 mg/kg. Fetal survival was not affected at 125 mg/kg BA. Despite decreased appetite in the infusion animals throughout gestation, mean litter size and fetal weights were comparable to the control animals. These results (for BA) were consistent with the published literature and thus demonstrated that the laboratory’s procedures and evaluation methods were suitable for identification of BA-induced effects (part i) as well as early gestational maternal manipulation (part ii) and gestational infusion (part iii) on embryofetal development and pregnancy outcome in NZW rabbits.
853 LIPID MEDIATORS LINK CELL CYCLE PROGRESSION WITH PLACENTAL AND NEURAL TUBE DEFECTS AFTER MATERNAL FUMONISIN EXPOSURE.

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Fumonisin B1 (FB1) is a mycotoxin produced by a common fungal contaminant of maize. Increased risk for neural tube defects (NTDs) is observed in populations that rely on maize as a dietary staple. FB1 inhibits ceramide synthase, resulting in altered pools of biologically active sphingolipids. FB1 (20mg/kg/day ip, E7.5-8.5) was administered to pregnant LM/Be and SW mice. On E9.5, maternal blood, embryonic and placental tissue was collected for LC-ESI-MS analysis of sphingolipid metabolites. Embryos/placentas were processed for histology, and TEM/SEM observation of neuroepithelial ultrastructure. Neural tissue from E9.5 control and FB1-exposed embryos was collected for Affymetrix microarray analysis. Maternal FB1 resulted in placental abnormalities and NTDs in the LM/Be strain. Elevated levels of sphinganine (Sa), 1-deoxy-Sa, and Sa-1-phosphate (Sa1P) were measured in maternal blood and placental tissue. 1-deoxy-SA is known to cause cell cycle arrest and Sa1P functions as a ligand for G protein-coupled S1P receptors (known modulators of lymphocyte chemotaxis). FB1 exposure altered migration of uterine NK cells and increased differentiation of giant trophoblast cells. The neuroepithelial expression of major secretory proteins, cell cycle, ciliogenesis, and cell cycle progression was measured in maternal blood, placenta, and developing embryo. Lipid mediators play important roles in chemotaxis and cell cycle progression. Primary cilia are involved in maternal blood, placenta, and developing embryo. Lipid mediators play important roles in chemotaxis and cell cycle progression. Primary cilia are involved in lipid metabolism. Lipid mediators play important roles in chemotaxis and cell cycle progression. Primary cilia are involved in lipid metabolism.

854 IS REDUCED FOOD CONSUMPTION A PREDICTOR OF ABORTIONS IN RABBITS?

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Increased abortion rates and reduced fetal weights are generally observed when maternal food consumption (FC) is severely restricted during organogenesis. However, rabbits usually tend to consume less food when treated during pregnancy; an effect that is most likely related to the stress of handling. The objective of this evaluation was to elucidate the effects of reduced FC as a good indicator of early viability and growth processes. In this study, the results of this study revealed a differential expression of genes involved in cell cycle progression, ciliogenesis, and Wnt signaling; all three are involved in chemotaxis and cell cycle progression. Primary cilia are involved in migration and assembly/disassembly of the primary cilium is coordinately regulated with cell cycle. Giant trophoblast cells differentiate by exiting the cell cycle at G2/M. Elevated levels of Sa1P and 1-deoxy-Sa may therefore impact signaling pathways involved in uNK cell migration, cell cycle progression, giant trophoblast cell differentiation, ciliogenesis, and neural tube closure.

855 ARRAY ANALYSIS OF LASTING BRAIN EFFECTS FROM PERINATAL PESTICIDE EXPOSURE.

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Chlorpyrifos (CPF), a widely used organophosphorus pesticide that irreversibly inhibits acetylcholinesterase (AChE), is thought to affect immature nervous systems adversely, but long term effects of this compound at low levels have been little studied. To explore this issue we launched studies to examine delayed effects of perinatal CPF on the expression of all genes represented on the Affymetrix Rat Genome 230 2.0 microarray. Time-pregnant Long-Evans rats were gavaged with clinically subtoxic doses of CPF in corn oil (12.5, 2.5, or 0.5 mg/kg) from gestational day 7 to postnatal day 21. Brains were collected later from progeny reaching early adulthood (day 101), and RNA was isolated, processed, and applied to the microarrays. These experiments are still in progress but initial data provide striking evidence that perinatal CPF exposure leads to altered expression in multiple gene pathways. The dams treated with 2.5 mg/kg did show significant inhibition of plasma AChE (66 ± 10%) but there were no clinical signs (e.g., tremors, fasciculation or salivation) even at the highest dose level. The array data from the 2.5 mg/kg group indicated a substantial number of altered pathways. These pathways were not obviously related to cholinergic function, as might have been expected from the primary mechanism of acute CPF toxicity. The most significant changes were in pathways grouped under "molecular signaling", including those for "hemostasis" (GO 7599) and "muscle contraction" (GO 6936); those under "inflammatory response", including "cell killing" (GO 1906); those under RNA metabolism (GO 16070); those under "cycloleucine metabolism" (GO 9187); and those under "mitochondrial function", including "mitochondrial membrane" (GO 31966). Proteomics studies to confirm the array results are in progress. Nonetheless these findings indicate that CPF can exert persistent effects on the developing rodent brain and they raise concerns about the potential for such effects in human infants.

856 EXTENDED ONE-GENERATION REPRODUCTION STUDY WITH METHIMAZOLE AS A REFERENCE CHEMICAL.

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The extended one-generation reproduction study (Cooper et al., 2006) is a complex and logistically-demanding study type, designed to assess potential effects of chemicals on pre- and postnatal development, reproductive function, neurologic and immunologic function and systemic toxicity in a single study. This study design incorporates a compliment of tests that are included for use in risk assessments and to determine whether further investigation is warranted. While the elements included in this new study type have been used in other guideline studies (e.g., reproduction, developmental neurotoxicity and developmental immunotoxicity), this new protocol requires vetting to verify feasibility to perform the study and verification the tests will detect critical effects (e.g., reproductive, neuro- and immuno-toxicity). This study tested the anti-thyroid drug methimazole as a reference chemical for thyroid endpoints, delayed development and neurotoxicity. Methimazole (99.2% purity) was administered continuously via the diet to Wistar (Han) rats (30/sex/dose level) at concentrations of 0, 15, 30 or 45 ppm, from 4 weeks prior to mating through termination of the F1 generation (approximately postnatal Day 75). Effects on P-generation males and females included increased plasma TSH and thyroid weight and reduced body weight at 30 and 45 ppm. There was no effect on reproduction, pup birth weight or pup viability at any dietary level. Dose-related effects on the F1 males and females included decreased body weight gain during lactation and after weaning, characteristic effects on thyroid endpoints, delayed sexual maturation, delayed sexual maturity, decreased sexual maturation. These results support the sensitivity of this study type to identify effects on thyroid, delayed development and the nervous system.

857 FEASIBILITY OF THE FI-EXTENDED-ONE GENERATION REPRODUCTION TOXICITY STUDY.

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The Extended One-Generation Reproduction Study (Cooper et al, 2006) was conceived as part of efforts to streamline toxicity testing and reduce the use of animals, and is now being developed as an OECD test guideline. The study is intended to assess potential effects of chemicals on development, reproduction, neurologic and immunologic function and systemic toxicity in an integrated manner. By enhancing the duration and extent of Fi offspring assessment, a 2nd generation can be waived in most cases. The purpose of this work was to evaluate the technical feasibility of this study design using reference chemicals: vinclozolin (reproductive/endocrine), methimazole (anti-thyroid, developmental neurotoxicity), and lead ac-
858 CATALASE IN THE MECHANISM OF METHANOL DEVELOPMENTAL TOXICITY.

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Oxidative stress and reactive oxygen species (ROS) have been implicated in the developmental toxicity and potential carcinogenic effects of methanol (MeOH) in rodent models, but the mechanisms remain unclear, particularly with regard to catalase, which in rodents both detoxifies ROS and metabolizes MeOH and its formic acid (FA) metabolite. To explore the role of ROS and catalase in MeOH teratogenicity, we are using genetically modified mouse strains that either express high catalase activity (transgenic mice expressing human catalase, Tg hCat) or low activity (mutant acatalasemic mice, Acat). Pregnant mice were treated on gestational day (GD) 8 with two doses of either MeOH (2 g/kg ip, 20% [w/v] solution in normal saline, given 4 hr apart, for a total of 4 g/kg), or its saline vehicle (GD) 8 (plug date = GD 1) with two doses of either MeOH (2 g/kg ip, 20% [w/v] solution in normal saline, given 4 hr apart, for a total of 4 g/kg), or its saline vehicle.

In preliminary studies, MeOH exposure most commonly caused ophthalmic abnormalities, with an overall incidence of 40–50% in Tg hCat and wild-type (WT) mice for both strains, and 20% in Acat mice, compared to <10% in all saline controls. The overall incidences for NTDs (seen only in WT for Acat strain and cleft palate (seen only in Tg hCat and their WT) were 12% and 10-13% respectively with MeOH exposure, compared to 0% and <3% in saline controls. At this preliminary stage, the limited number of litters and fetuses in several genetic groups precluded a statistical analysis of the differences among genotypes. If current trends are confirmed in ongoing studies, the apparent respectively enhanced and reduced susceptibilities of Tg hCat and Acat mice would suggest that the role of maternal and possibly fetal catalase in MeOH and/or FA metabolism is more developmentally important for MeOH exposure than its role in fetal ROS detoxification. (Support: Methanol Foundation, CIHR)

859 IN UTERO BISPHENOL A EXPOSURE MAY CAUSE STRUCTURAL AND FUNCTIONAL CHANGES IN THE DEVELOPING MURINE NERVous SYSTEM.

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The endocrine disruptor bisphenol A (BPA), found in polycarbonate plastics and epoxy resins, is a potential human toxicant, with a rodent LOAEL of 50 mg/kg/day (U.S. EPA). However, this estimate may not reflect all species and alternate mechanisms, such as production of reactive oxygen species (ROS), occurring at lower exposures. This was investigated in pregnant C57BL/6J mice treated i.p. on gestational day 15 with BPA (100 mg/kg or 100 mg/kg), methamphetamine (METH) (40 mg/kg) as a ROS-initiating positive control, or their respective vehicles. Pups were evaluated for histopathological changes in the developing brain 6 hr post-injection, and for cognitive deficits at 4-6 weeks of age. In preliminary studies, B-tubulin staining revealed an apparent dispersed pattern of axon innervation within the cortical plate of BPA-exposed mice at both doses, and disruption of the laminar organization in the cortical plate-marginal layer interface. A trend for increased phosphorylated histone H3-positive neuroprogenitor cells was observed in the subventricular zone of the neocortex with low-dose BPA. Preliminary studies in a limited number of mice using the olfactory discrimination test (ODT) suggested a potential reduction in sensory detection with METH and high dose BPA, but the doses appeared to be too toxic for an evaluation of cognition. With low-dose BPA, preliminary ODT results suggested that olfactory discrimination reflecting cognition may be reduced. If confirmed in a full study, these preliminary results would suggest that in utero exposure to BPA at doses well below the EPA LOAEL may cause neurodevelopmental deficits in mice. (Support: CIHR, Doctoral Awards to AMS (CIHR Frederick Banting & Charles Best Award) and AR (CIHR R&ED Award))

860 SUBTHRESHOLD DOSES OF CADMIUM AND ARSENITE, BUT NOT OTHER METALS, COMBINE TO PRODUCE NEURAL TUBE DEFECTS IN C57BL/6J MICE.

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Sponsor: D. Howland.

Synergistic interaction among teratogens may be mechanism dependent. If true, this would suggest that only a subset of teratogens would have the capacity to interact in this way and that previous studies of teratogens selected based on other considerations might miss this phenomenon. The present study examines whether subthreshold doses of environmentally common, teratogenic metals could combine to exceed the threshold for neural tube defects (NTDs). First, NTD subthreshold and suprathereshold doses were established in C57BL/6J mice (gd 8.0; ip for arsenic, cadmium, mercury, nickel, and zinc). Subthreshold doses did not cause NTDs, significantly reduce fetal weight or significantly increase resorptions. Next, subthreshold doses were administered in combination. The majority of combinations demonstrated no additivity or synergism in the production of NTDs, even one that showed strong synergism with respect to maternal toxicity. The combination of As and Cd, however, was synergistic with respect to the production of NTDs (35% NTDs with subthreshold doses) and resorptions. We have previously shown that As and Cd exhibit the opposite strain sensitivity in C57BL/6J and SWV mice compared to other NTD-inducing agents. Taken together, these data support the hypothesis that additivity, as well as strain sensitivity, in the production of NTDs is mechanism dependent. Thus, while many subthreshold teratogens may not interact to exceed a threshold for the production of a congenital malformation, at least some do, and these merit further study as to mechanism of action. DNA microarrays have identified candidate genes that may be involved in the interaction between As and Cd on the developing nervous system.

861 ACTIVATION OF ARYL HYDROCARBON RECEPTOR SIGNALING IS ASSOCIATED WITH DOWN REGULATION OF SLUG DURING MOUSE PROSTATE DEVELOPMENT AND TRANSGENIC ADENOCARCINOMA OF THE MOUSE PROSTATE (TRAMP) TUMOR METASTASIS.

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Development of C57BL/6J male fetal prostate is impaired by the aryl hydrocarbon receptor (AHR) ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Metastasis of C57BL/6J x FVB transgenic adenocarcinoma of the mouse prostate (TRAMP) tumors is impaired by the AHR ligand 6-methyl-1,3,8-trichlorodibenzo-p-dioxin (6-MCDF). This study is an initial attempt to test the hypothesis that these AHR-related outcomes are triggered by the common mechanism of impaired epithelial cell migration. Since down regulation of e-cadherin has been associated with invasive growth in human prostate cancer we first sought to determine whether this event also occurs during early prostate development in control mice. Immunohistochemical analysis of embryonic day (E) 17.5 male fetal mouse urogenital sinus (UGS) indicated focally decreased e-cadherin protein levels in basal epithelial cells located in the tips of nascent prostate ducts (prostatic buds). Down regulation of e-cadherin at bud tips was accompanied by up regulation of the e-cadherin transcriptional repressor, SLUG. Exposure to TCDD (5 μg/kg, maternal dose) on E15.5 impaired prostatic bud formation, prevented the down regulation of e-cadherin in UGS basal cells, and significantly decreased the number of SLUG-immunopositive basal cells in the UGS. We next evaluated SLUG protein abundance in TRAMP tumors from control mice and mice that were fed 6-MCDF (40 mg/kg diet). We showed previously that this 6-MCDF dose significantly impaired prostate tumor metastasis and we show here that it also results in a major reduction in SLUG protein abundance. These findings suggest that down regulation of SLUG protein abundance may be a common mechanism for inhibition of prostatic bud outgrowth by TCDD and inhibition of TRAMP tumor metastasis by 6-MCDF (Supported by NIH grants ES01332 and DK083425).
To protect children against exposure to chemicals in the environment the law demands safety testing including mandatory research in animals (mainly rats). Guideline studies to test developmental neurotoxicity involve daily testing of hundreds of animals to reflect on their development. This behavioral survey during the pre-weaning phase requires intensive handling of the pups to study the development of senses and reflexes. Although not invasive, these activities greatly disturb normal housing and nursing of the litter in the home cage. We argued that at least some of this tedious testing could be replaced by an animal friendly marker with high discriminative power. Here, we studied the Ultrasonic Vocalizations (USVs) of the rat pups, i.e. calls of 35–65 kHz emitted by rodent pups to communicate with their mothers. USVs in rat pups were counted during 30s each day, from postnatal day 4 to 18. The mother animals were exposed daily (gestation day 6 to factation day 10) to different doses of known developmental toxicants (MeHg, DOTC and TBTO). The question was raised whether: 1) USVs could serve as a marker to study normal development and 2) as a marker to study developmental (neuro)toxicity. The results showed that during normal development the number of calls emitted by the pups typically changed over time from day 4 to 18 postnatally, with a peak around day 12. Unlike DOTC (a developmental immunotoxin), TBTO and MeHg (both developmental neurotoxicants) clearly affected the normal developmental pattern of the USVs by reducing their number and/or delaying their onset. The frequency of the calls, although also slightly affected, appeared a less sensitive indicator. Together, these results support the suggestion that USVs form a fast, sensitive and animal-friendly marker to study normal neural development and developmental neurotoxicity and warrant a role for USVs in developmental safety testing in time.

Reduced Ethanol Embryopathies in Embryo Culture in Transgenic Mice Expressing Human Catalase.

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Reactive oxygen species (ROS) have been implicated in the mechanism of the Fetal Alcohol Spectrum Disorder (FASD) caused by in utero exposure to ethanol (EtOH). Although the antioxidant enzyme catalase may protect the embryo by detoxifying ROS, it also metabolizes ethanol to acetaldehyde, which may be embryotoxic. The involvement of ROS was investigated in whole embryo culture to remove maternal factors and determine the role of embryonic catalase in ethanol embryopathies. C57BL/6J mouse embryos expressing human catalase (hCat) or their wild-type controls were explanted on gestational day (GD) 9 (plug = GD 1), exposed for 24 hr to 4 mg/mL EtOH or vehicle and evaluated for functional and histological endpoints. The hCat embryos were protected from the embryopathic effects of EtOH, with at least 90% survival of hCat embryos in contrast to only 10% survival of their wild-type controls. In addition, hCat embryos showed reduced expression of delayed Neuropore closure compared to wild-type controls. This suggests that ROS may be involved in the mechanism of the Fetal Alcohol Spectrum Disorder and that catalase activity may be a determinant of FASD risk. (Support: CIHR)

Sexing of Early Postimplantation Rat Embryos by Amplification of Sry Gene in Stored 2-DE Samples for Developmental Toxicity Studies.

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Some chemicals show gender differences in developmental toxicities when examined in rat fetuses or neonates. However, developmental toxicity studies of chemicals with early postimplantation rat embryos both in vivo and in vitro have often been performed irrespective of their gender. This is because there are no visible gender characteristics, although sex determination and gonadal differentiation begin around this developmental stage in rodent embryos. Proteomics analyses are the exhaustive study of proteins, identifying protein changes accompanying changes in biological function. We have shown the usefulness of proteomics analysis by two-dimensional electrophoresis (2-DE) in cultured postimplantation rat embryos as the approach to mechanistic investigation of developmental toxicity by chemicals. In this study, we explored sexing of rat embryo stored in frozen 2-DE samples by polymerase chain reaction (PCR) of a male-specific gene sequence, Sry. The embryo proper and yolk sac membrane at gestation day 11 from Wistar rats were used for stored embryonic 2-DE samples. The embryonic 2-DE samples were desalted and their total DNA was extracted. The Sry sequence in the extracted DNA was amplified by PCR and the product was analyzed by agarose gel electrophoresis. A sequence of beta actin gene (Actb) was also amplified simultaneously as a control. The embryos with the PCR product of Sry were determined as male, and those without the product were determined as female. It was concluded that stored embryonic 2-DE samples could be used for retrospective examination of gender-related effects in proteomic analysis of developmental toxicity.
Melatonin Ameliorates Cyclophosphamide-Induced Trans-Placental Genotoxicity, Germ Cell Toxicity and Urototoxicity in Rat: Role of Nuclear Erythroid 2-Related Factor 2 and Nuclear Factor-KappaB.


The present study was aimed to investigate the protective effects of melatonin against cyclophosphamide (CP) induced trans-placental genotoxicity, germ cell toxicity and urototoxicity. Further attempt has been made to study the effect of melatonin on the transcription factor nuclear factor E2-related factor 2 (Nrf2) and Nuclear factor kappa-B (NF-kB). In trans-placental genotoxicity study CP and melatonin were administered in the late gestation period (day 15 to day 20) and all the pups were sacrificed within 24 hours after their birth. In germ cell toxicity study CP was administered once in a week for 5 consecutive weeks and melatonin was administered 5 days in a week for 5 consecutive weeks. However, in urototoxicity study melatonin was administered 3 day prior and one day after the administration of CP. Melatonin prevents CP-induced trans-placental genotoxicity as observed from comet assay parameters performed in bone marrow, lymphocytes, liver and kidney cells as well as reduced micronuclei count in peripheral blood cells of newborn mice. The protective effect of melatonin against CP-induced male germ cell toxicity was observed by restoration in body weight, testes and epididymis weight, sperm count, sperm head morphology, sperm comet assay, histology of testes and TUNEL assay parameters. Further, melatonin inhibits the CP-induced urototoxicity as observed by reduction in oxidative stress, DNA damage and histology of the bladder. Treatment with melatonin led to increased expression of Nrf2 and decreased expression of the NF-kB in the bladder tissue of rat. In conclusion inter-vention of melatonin ameliorates CP induced trans-placental genotoxicity, germ cell toxicity and urototoxicity. The activation of transcription factor Nrf2 and repression of NF-kB plays major role in the protection offered by melatonin.

A Dosimetry Model for Uptake of Inhaled Soluble Vapors in the Human Lower Respiratory Tract.

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Current vapor dosimetry models rely on the numerical solution of the governing convective-diffusion equation, which introduces artificial diffusion and can be computationally expensive. As a result, in practice these models are applied to simplified lung geometries such as a typical-path model. A new, lung dosimetry model for inhaled vapor uptake was developed based on an existing model for nanoparticle deposition. Analytical solutions of vapor losses were obtained during the inhalation, pause, and exhalation phases of the breathing cycle, thereby avoiding intensive numerical computations. Vapor uptake was calculated for formaldehyde (a highly soluble gas, used as a positive control for model validation). Inhaled vapors penetrated through the bronchial tree with very little reaching the alveolar region of the human lung. Airway extraction efficiency for a minute ventilation of 7.5 LPM exhibited a steady drop during the first 4 airway generations but increased to a peak in generation 9, followed by a sharp decline to vanish just past the terminal bronchioles. The mass flux was highest in the trachea but decreased sharply over the length of the conducting tree until vanishing at the entrance to the respiratory bronchioles. Over 94% of inhaled vapors were eliminated in the bronchial tree with the rest exiting the lung on exhalation. The proposed model, due to its short execution time, can be used to predict losses in the lungs of humans and laboratory animals. In addition, it can be coupled with available pharmacokinetic models to determine the disposition of inhaled gases throughout the body. This study was funded by Research Institute for Fragrance Materials, Inc.
VALIDATION OF COMPUTATIONAL PREDICTIONS OF AIRFLOWS AND 3D VELOCITY FIELDS IN THE RODENT LUNG WITH 3HE MRI AND FLUORESCENT MICROSCOPE DEPOSITION.

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3D Computational fluid dynamics (CFD) models of the respiratory system are increasingly used to improve predictions of site-specific dosimetry of a variety of inhaled materials. CFD models can improve the design of drug delivery systems as well as facilitate the design, interpretation, and extrapolation of animal study results to humans. Kimbell and colleagues have extensively validated 3D/CFD models for nasal airways. However, expanding and contracting tissues in the lung complicates the resolution that can be achieved in validation studies. We have thus developed a hyperpolarized 3He MRI approach that allows us to determine both regional airflows as well as site-specific velocity fields in conducting airways of live animals. Computational grids were developed from micro-CT images of lung casts from each animal after MRI. Several levels of airflow truncation of the lung cast data were investigated to determine the potential impact of distal airflow resistance in CFD simulations. In a separate subgroup, animals were also exposed by inhalation to an atmosphere containing 1.0-micron fluorescent microspheres (FMS) followed by micro-dissection and analysis. The FMS and MRI analyses were tightly correlated for regional airflows. The CFD simulations, after accounting for the high diffusivity of 3He, were also able to match regional airflows as well as 3D velocity profiles. As a result of these validation studies, the uncertainty in predictions of localized dosimetry of airborne materials in the lungs and humans can be reduced. This experimental approach can also be used along with CFD models directly coupled with ODE models of tissue mechanics (resistance, compliance, elastance) to develop and validate dosimetry models for pulmonary disease conditions, an important condition for both drug delivery and risk assessments for potentially sensitive populations. Funded by NHLBI R01 HL073598.

MULTI-SCALE MODELING OF THE RODENT RESPIRATORY SYSTEM.

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Rigid, steady-state 3D computational fluid dynamics (CFD) simulations of nasal airflows have improved cross-species comparisons of site-specific dosimetry for a variety of inhaled materials. As these models extend to the lung and include the full breathing cycle, they transcend multiple geometric scales and must factor in the effects of airway resistance and tissue properties. Historically, one-way coupling of ODE models such as airway wall metabolism as a boundary condition in CFD models have been straightforward. However, tightly linked, two-way coupling of ODE with CFD models with iterative communication across scales that is critical for incorporation of tissue mechanics and their effects in disease remains a challenge. We therefore devised a novel nonlinear Krylov accelerator for accelerating Newton iterations between CFD and ODE models and further reduced cost by eliminating explicit evaluations of the Jacobian matrix by implicitly accumulating and updating Jacobian information on the active ‘Krylov’ subspace over multiple time steps. To demonstrate the approach, we dynamically determined outlet pressures for imaging-derived pulmonary geometries, given only alveolar pressure and atmospheric pressure. ODEs describing the distal airway tree consist of resistance and compliance and inertance. This new accelerator allows us to compute outlet pressures at each outlet and at each time step rather than assuming all airway outlets are at the same pressure as is currently done. Remarkably, due to reuse of the Krylov subspace, the computational cost of the PDE-ODE coupling is similar to standard CFD simulations with prescribed pressures at the outlets and is relatively independent of the number of outlets. To validate the method, we compared a 4-generation CFD Weibel model with a known solution with a 2-generation CFD-ODE model. The new accelerator now allows us to begin combining spatial (CFD) models with non-spatial (network ODE) models with little computational overhead and great flexibility. Funded by NHLBI R01 HL073598.

A GENERAL APPROACH FOR SPECIFYING INFORMATIVE PRIOR DISTRIBUTIONS FOR PBPK MODEL PARAMETERS.


Characterization of uncertainty in model predictions is receiving more interest as more models are being used in applications that are critical to human health. For models in which parameters reflect biological characteristics, it is often possible to provide estimates of parameters along with uncertainties even in the absence of experimental results against which to compare predictions. Thus, uncertainties for model predictions can be derived from such parameter uncertainty even when there are little or no in vivo data available. When appropriate data do exist, such prior information (or priors) can be incorporated into Bayesian statistical methods for parameter estimation. Informative priors are often used for physiological parameters in PBPK models to indicate how well-known these parameters are. However, chemical-specific parameters are often assigned vague or weakly informative priors due to much greater uncertainty in parameter values. We describe some approaches that can be used to specify more informative priors for chemical-specific parameters based on information obtained from computational predictors, such as QSAR models, that have been fully considered in risk assessment. The need to account for human variability in cancer susceptibility, other than possible early-life susceptibility, is increasingly evident to the scientific community and regulatory agencies. The overarching premise of this research is that apparent species- and organ-specific metabolism and toxicity of trichloroethylene (TCE) are genetically controlled and that mechanisms of susceptibility can be successfully elucidated using a genetically diverse panel of inbred mice. To that end, the Hack et al. (2006) mouse TCE PBPK model was adapted to describe the TCE metabolite data collected in a panel of 16 inbred mouse strains. The revised model successfully described the time-course disposition of trichloroacetic acid (TCA), dichloroacetic acid (DCA), s-(1,2-dichlorovinyl)glutathione (DCVG), and s-(1,2-dichloroethyl)-L-cysteine (DCVC) in B6C3F1 mice. Monte Carlo analysis was used to evaluate the variability in metabolism and clearance of the four TCE metabolites after a single oral bolus of TCE (2100 mg/kg) over a 24 h period. Kinetic analysis of the relative flux of TCE metabolized through the GSH conjugative pathway (DCVG and DCVC) compared with the oxidative pathway (TCA and DCA) resulted in a 12-fold difference across panel of strains. Coefficients of variation on the order of 30 to 70% in the metabolic rate coefficient estimation. Informative priors are often used for physiological parameters in PBPK models to indicate how well-known these parameters are. However, chemical-specific parameters are often assigned vague or weakly informative priors due to much greater uncertainty in parameter values. We describe some approaches that can be used to specify more informative priors for chemical-specific parameters based on information obtained from computational predictors, such as QSAR models, that have been fully considered in risk assessment. The need to account for human variability in cancer susceptibility, other than possible early-life susceptibility, is increasingly evident to the scientific community and regulatory agencies. The overarching premise of this research is that apparent species- and organ-specific metabolism and toxicity of trichloroethylene (TCE) are genetically controlled and that mechanisms of susceptibility can be successfully elucidated using a genetically diverse panel of inbred mice. To that end, the Hack et al. (2006) mouse TCE PBPK model was adapted to describe the TCE metabolite data collected in a panel of 16 inbred mouse strains. The revised model successfully described the time-course disposition of trichloroacetic acid (TCA), dichloroacetic acid (DCA), s-(1,2-dichlorovinyl)glutathione (DCVG), and s-(1,2-dichloroethyl)-L-cysteine (DCVC) in B6C3F1 mice. Monte Carlo analysis was used to evaluate the variability in metabolism and clearance of the four TCE metabolites after a single oral bolus of TCE (2100 mg/kg) over a 24 h period. Kinetic analysis of the relative flux of TCE metabolized through the GSH conjugative pathway (DCVG and DCVC) compared with the oxidative pathway (TCA and DCA) resulted in a 12-fold difference across panel of strains. Coefficients of variation on the order of 30 to 70% in the metabolic rate coefficients were necessary to describe the variation in TCE metabolism across the panel of mice. The direction of this work is to further investigate the variability of response by strain by addressing the dose-response and additional time points to provide quantitative assessment of inter-individual genetic variation with important implications for human population dose-response profiles of TCE susceptibility.

MONTE CARLO ANALYSIS OF VARIABILITY IN TCE METABOLISM ACROSS A PANEL OF INBRED MOUSE STRAINS WITH A PBPK MODEL.

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Genetic polymorphisms can profoundly affect individual differences in susceptibility to disease after exposure to environmental agents. Yet, these factors have not been considered in epidemiology. It is critical that we use computational models to understand species-wide effects of site-specific factors on immune response in vivo. To improve understanding of the genetic basis of variability in TCE metabolism across a genetically diverse panel of inbred mouse strains, we compared the in vitro and in vivo TCE metabolic profiles from 16 mouse strains. This work was reviewed by EPA and approved for publication but does not necessarily reflect official agency policy.

PROGRESS IN DEVELOPMENT OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR AVIATION FUELS.

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Military aviation fuels are complex mixtures of hydrocarbons in which the n-alkanes are prominent components. The pharmacokinetic behaviors for the majority of fuel constituents have not been described in the framework of physiologically-based...
A Physiologically Based Pharmacokinetic (PBPK) model for chloroform was developed as an exposure assessment tool. The four compartment model included the capacity to evaluate all relevant exposure pathways (inhalation, ingestion, and dermal). The utility of the chloroform PBPK model was evaluated by comparing internal dose estimates with a traditional exposure assessment. Exposure to chloroform in potable water was evaluated with a site-specific interest in potential chloroform exposures to the fetus. Also of interest was an assessment of the public health intervention strategy of supplying bottled water to the community as a means of mitigating the exposures. Comparisons were made by assessing chloroform exposure through inhalation during showering, dermal absorption during showering, and ingestion. The traditional exposure assessment predicted that substituting bottled water for the household potable water would reduce the total external chloroform dose by 45%. PBPK modeling predicted an internal dose (AUC) reduction by using bottled water of only 28%. Perhaps more importantly, the comparisons of the PBPK model and the traditional exposure assessment suggested the majority of the chloroform exposure was associated with inhalation during showering, and that providing bottled water reduced the maximum chloroform blood level to the fetus by only 8%. With regard to fetal exposures, the PBPK modeling suggests that mitigation efforts that address chloroform (and perhaps other VOCs) exposures related to inhalation would provide a larger exposure reduction for the fetus.

A Physiologically-Based Pharmacokinetic (PBPK) tool kit to address public exposures to environmental pollutants.

### Tissue Partition Coefficients for Nonane and Its Isomers.

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JP-8 is a major fuel source used by US and NATO forces. JP-8 is a complex mixture of aliphatic and aromatic isomers of hydrocarbons. Occupational exposure to JP-8 occurs through inhalation and dermal contact. Physiologically based pharmacokinetic models are being developed to predict the target organ dosimetry and body burden for jet fuels for rodents used in toxicity testing. Tissue/blood partition coefficients (PCs) values are chemical specific parameters used in modeling. The partition coefficient values for n-alkanes tend to increase with the increasing carbon number but less is known about the trend for isomers of n-alkanes. Fisher F344 rat tissues were used to experimentally measure the tissue to air partition coefficient values for liver, lung, muscle, fat, brain and whole blood. PCs were determined by vial equilibration methods which was developed by Sato and Nakajima (1979) and later modified by Gargas et al. (1989). PCs were obtained for five isomers of n-alkane nonane (C9), namely 3-methylnonane, 4-ethylheptane, 2,3,5-trimethylheptane, 2,2,4,4-tetramethylhexane and 2,2,4,4-tetramethylpentane and n-nonane. The PC values tend to follow the published log octanol/water (O/W) PC values for nonane and its isomers. That is experimentally determined PC values n-nonane with the highest O/W value were greatest and the isomer 2,2,4,4-tetramethylpentane with the lowest O/W were the lowest. As expected the fat tissue had the highest EST PC values for n-nonane and the isomers and muscle the least. Five replicates of sample and three replicates of reference vials were made for each tissue for each chemical and the coefficient of variation ranged from 1 to 15 percent. These reported PCs will support the development of a jet fuel PBPK model.

### A Physiologically-Based Pharmacokinetic Model of Endotoxin from Salmonella Typhimurium in the Rat Brain and Peripheral Organs.


We have previously measured the distribution and pharmacokinetics of biosynthetically labeled endotoxin of Salmonella Typhimurium with 3H (fatty acid chains) and 14C (glucosamine residues). Rats were administered saline containing 14C, 3H-LPS (200 µg/kg) intraperitoneally (IP). To predict the dynamics of endotoxin exposure, we developed a physiologically-based pharmacokinetic model in the Sprague-Dawley rat using our measured data and model results. The results showed adsorption and biphasic decay over 48 hrs in plasma as well as tissue accumulations of the fatty acyl chains and glucosamine residues in various target organs, including the brain. We also found that the glucosamine and fatty acyl components separated in vivo about 4 hr after IP injection. Each component had its own dynamic behavior and target distribution in the rat. The fatty acyl components tended to remain in the brain stem, caudate nucleus, frontal cortex, hypothalamus, cerebellum, and hippocampus. Other organs (kidney, spleen, choroid plexus, meninges) had similar biphasic distribution. The liver had the unique accumulation of both glucosamine and fatty acyl residues.
plasma protein binding and ionization. The objective of the present study was to develop a unified algorithm such that the PCs for both drugs and environmental chemicals could be predicted. The resulting algorithm calculates PCs as the ratio of the sum of free and bound (i.e., nonspecific and specific) chemical in tissue components (intracellular and interstitial spaces) to that in blood (plasma and erythrocyte). In turn, the partitioning into each tissue or blood component was computed on the basis of the fractional content of, and partitioning into, the neutral lipids, proteins and phospholipids (neutral and acidic). The chemical or drug partitioning into these tissue fractions was calculated using available in vitro data on: (i) log P for neutral lipid:water PC, (ii) Fup for plasma protein:water PC, and (iii) blood:plasma ratio for hemoglobin:water and acidic phospholipid:water PC. The drug ionization in the aqueous phase of each fraction was also taken into account. The unified algorithm was then applied to calculate the Pb or Kpu for muscle (n = 153), liver (n = 155) and fat (n = 147) of acidic, neutral, zwitbornic and basic drugs as well as ketones, acetate esters, alcohols, aliphatic hydrocarbons, aromatic hydrocarbons and ethers. The resulting algorithm adequately reproduced the literature data on 455 PCs for these drugs and chemicals, and the level of prediction accuracy is identical to that of the previously published algorithms for specific sub-sets of chemicals or drugs. Overall, the present algorithm uniquely facilitates the computation of macro and micro level PCs for developing organ and cellular-level PBPK models for both chemicals and drugs (Supported by AFSSET, NSERC).

881 RELATIONSHIP BETWEEN INPUT PARAMETERS OF PHYSIOLOGICALLY-BASED (PB) PHARMACOKINETIC (PK) MODELS AND MACRO-CONSTANTS OF COMPARTMENTAL PK MODELS: A CASE STUDY WITH STYRENE.

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PBPK models are often preferred to strictly compartmental PK models due to their mechanistic nature and extrapolative potential. However, PBPK models require estimates of parameters that are not always easily accessible. For certain chemicals, empirical models that fit experimental data to a series of exponential terms do exist. However, little attention has been given to parameter translation between these two model types. The objectives of this study was to show the relevance of PBPK models for these tissues/organs absorb chemicals over time (dosimetry). The data these models provide are quantitatively akin to what one could “see” using whole-body autoradiographic studies performed on animals or humans in vivo. In this spirit, we describe and demonstrate a platform independent web-accessible tool PAVA (Physiological and Anatomical Visual Analytics) that has been used to map biological data onto either human or mouse anatomy and physiology. The type of data or information this visualization method may provide include (but are not limited to): (1) simulated and experimental tissue dosimetry (2) tissue specific gene expression (3) temporal changes in % organ weight (4) changes in organ-specific chemical partitioning as a function of chemical space descriptors (lipophilicity), (5) visualization of whole-body autoradiographs, (6) visualization of epidemiological data. We demonstrate for each of these data-types how PAVA can be of use. With the rise in use of visual analytics for knowledge mining of multi-dimensional data, such as biologically relevant data, the advantages that these visualization methods afford pay homage to the saying that a picture is worth a thousand charts. This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect official Agency policy.

882 THE CHEMICAL LANDSCAPE OF EXISTING PBPK MODELS AND ITS OVERLAP WITH AVAILABLE OPEN-ACCESS CHEMICAL DATABASES.


Physiologically based pharmacokinetic (PBPK) models have been applied for a wide variety of compounds to assess internal tissue concentrations in relation to administered dose for human health risk assessment. These models integrate information from physiology, chemistry, and biochemistry to deterministically simulate absorption, distribution, metabolism and elimination processes within an organism. A major challenge associated with PBPK model development is the inability to generally translate from pre-clinical and validated provisional models “on the fly”. Surveying the chemically-relevant landscape of available PBPK models will aid in identifying chemical data gaps to enhance our understanding and utilization of new techniques to provide better tools for the risk assessment community. Using bibliometric analysis similar to one employed on a recent study (Petrson et al., 2009), a retrospective literature survey was performed to identify PBPK-related articles up to 2009. There are approximately four hundred PBPK-related articles in existence yet there are far less chemically-unique PBPK models in the literature. The current work presents an in-depth analysis of the current physiochemical landscape upon which published PBPK models exist. These findings are then compared with the three national chemical databases utilized heavily within the risk assessment field — (i) CDC’s National Health and Nutrition Examination Survey (NHANES); (ii) USDA’s Pesticide Data Program (USDA-PDP); and (iii) U.S. EPA’s ToxCastTM to determine overlap, gaps and weaknesses in the available PBPK inventory. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

883 WHOLE-BODY VISUALIZATION OF PHYSIOLOGICALLY-RELEVANT DATA: OF MICE AND MEN.


The Exposure Dose Research Branch of the U.S. EPA’s National Exposure Research Laboratory has been developing various approaches for quantitative modeling of biological disposition of human-produced chemicals in support of chemical risk management. Often, Physiologically-Based Pharmacokinetic (PBPK) modeling approaches are used to simulate chemical-specific absorption, distribution, metabolism and elimination (ADME) processes that provide information on how tissues/organs absorb chemicals over time (dosimetry). The data these models provide are quantitatively akin to what one could “see” using whole-body autoradiographic studies performed on animals or humans in vivo. In this spirit, we describe and demonstrate a platform independent web-accessible tool PAVA (Physiological and Anatomical Visual Analytics) that has been used to map biological data onto either human or mouse anatomy and physiology. The type of data or information this visualization method may provide include (but are not limited to): (1) simulated and experimental tissue dosimetry (2) tissue specific gene expression (3) temporal changes in % organ weight (4) changes in organ-specific chemical partitioning as a function of chemical space descriptors (lipophilicity), (5) visualization of whole-body autoradiographs, (6) visualization of epidemiological data. We demonstrate for each of these data-types how PAVA can be of use. With the rise in use of visual analytics for knowledge mining of multi-dimensional data, such as biologically relevant data, the advantages that these visualization methods afford pay homage to the saying that a picture is worth a thousand charts. This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect official Agency policy.

884 ASSESSING THE TRACER KINETICS OF MANGANESE IN MONKEYS AND HUMANS WITH PBPK MODELING.

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Manganese (Mn), an essential element, is naturally present in food, water, and airborne dusts. Human health concerns focus on central nervous system toxicity which may occur from inhalation exposure to high concentrations of Mn particulates in dusty trades and some welding operations. Risk assessments for inhaled Mn should consider endogenous Mn levels from dietary absorption and homeostatic controls that regulate tissue concentrations despite wide fluctuations in daily Mn intake. Tracer studies permit assessment of overall kinetic behavior of compounds that are maintained in steady-state through continuous dietary intakes. We have applied physiologically-based pharmacokinetic (PBPK) models for Mn to evaluate expected tracer kinetics for various exposure scenarios in monkeys and humans. Model parameters for monkeys were calibrated using steady-state tissue Mn concentrations from dietary and inhalation exposures and were subsequently scaled for the human. The PBPK models developed for inhalation and oral uptake were modified to include intravenous (iv), intraperitoneal, and subcutaneous exposure routes.
Manganese (Mn) is a ubiquitous, essential element that can be neurotoxic upon overexposure. Although Mn neurotoxicity has most commonly been associated with inhalation of excessive amounts of Mn-containing fumes and dusts, it may occur upon over-exposure by other routes. Dose to target tissue, not exposure route, is the critical determinant in the development of Mn neurotoxicity. Recently, tissue Mn data from a series of pharmacokinetic studies were used to develop a physiologically based pharmacokinetic (PBPK) model for ingested and inhaled Mn in rats and non-human primates. The models demonstrated that dose-dependent transitions exist for tissue accumulation of ingested Mn, due in part to homeostatic regulation. The purpose of this study was to use these PBPK models to improve on traditional human health risk assessment approaches for environmental exposures to Mn. The PBPK models were used to estimate chemical-specific adjustment factors (CSAFs) in lieu of relying on default uncertainty factors (UFS). CSAFs were calculated for pharmacokinetic differences among potentially susceptible subpopulations and life stages. These comparisons included gender, and age, as well as fecal and neonatal life stages. The resulting CSAFs were then used to replace some of the default UFS that have been used in risk assessments for ingested Mn based on neurological effects observed in occupational studies in the derivation of an acceptable environmental exposure concentration. Monte Carlo analysis demonstrated that the variation in target tissue dose at the resulting environmental exposure concentration was less than that associated with normal dietary variation. This work shows how PBPK models can be used to produce more robust and biologically based risk assessments of essential elements such as Mn by accounting for innate homeostatic control processes, dose-dependent transitions for tissue accumulation, and population variability.

**TARGET-TISSUE DOSIMETRY MODELING TO SUPPORT THE RISK ASSESSMENT OF MANGANESE.**

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Workload has been recognized as a major determinant of the absorbed dose for many solvents. This study was undertaken to assess the impact of physical exertion (workload) on the biological levels of unchanged styrene (STYR) or STYR metabo- lites used as biological exposure indices (BEIs). Physiologically based toxicokinetic models were adapted and validated in order to simulate a typical weekly occupational exposure (8/5/3 days) to STYR alone and combined with acetone (ACE) at their current threshold limit values (ACGIH) of 20 ppm and 100 ppm, respec- tively. Simulations were then conducted under workload levels corresponding to rest (12.5W), 25W and 50W, and the impact on the levels of STYR in venous blood (STY-B) and on urinary mandelic (MA) and phenylglyoxylic (PGA) acids at the end of the last work shift of a week was examined for a typical worker. The predicted values were compared to results of both experimental and field studies which supported the adoption of the current BEIs for STYR. For an exposure to 20 ppm, the end-of-shift values of STY-B for a workload of 50W showed a 3-fold increase compared to the value at rest (0.17 mg/L), whereas the sum of MA and PGA in urine was 2.7 times higher than at rest (144 mg/g creatinine). The model predicted slight effect of co-exposure to ACE on biological levels of STYR at these exposure levels. Based on the relation between physical activity and the values of BEIs predicted by the model, the average workload level in field studies was approximately 50W. Overall, the model described well the impact of workload on biological levels of STYR and showed that workload needs to be taken into account to avoid under-estimation of the internal exposure of workers and health risk. (Supported by AFSET, France and IRSST, Canada)

**APPLICATION OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL OF TRICHLOROETHYLENE IN RATS FOR ESTIMATION OF INTERNAL DOSE.**


Potential human health risk from chemical exposure must often be assessed for conditions for which suitable human or animal data are not available, requiring extrapolation across duration and concentration. The default method for exposure-duration adjustment is based on Haber’s rule which states that a constant toxic effect (K) is a function of exposure concentration (C or Cn) and exposure duration (t), K = C or Cn (or C)/t). This approach has been criticized for poor predictability, with errors increasing as the extrapolation interval increases. The purpose of the present work is estimation of the internal doses that result from various exposure concentrations and duration using a PBPK model developed in our laboratory for trichloroethylene (TCE) (Simmons et al., 2005). The model compartments are liver, brain, fat, richly-perfused and slowly-perfused tissues. TCE is a volatile organic compound and a common environmental pollutant of air, water and food. Mortality data from experimental animals are used in setting acute exposure guideline level-3 (AEGL-3) values as these represent air concentrations above which exposure could result in life-threatening adverse health effects or death (NRC, 2001). We compared results from simulations of two inhalation exposure scenarios reported to cause 50% mouse mortality (LC50) in rats, 26,000 ppm for one hour and 12,000 ppm for four hours. These resulted in simulated arterial blood concentrations at the end of the exposure period of 888 and 733 mg/L, respectively. While there was a 4-fold difference in exposure duration and a 2.17 fold difference (percent difference, 74%) in external exposure concentration, there was only a 1.2-fold difference (percent difference, 19%) in estimated arterial blood concentrations. This highlights the utility of PBPK modeling for estimation of internal dose when considering the health implications of exposures of varying duration and concentration and for extrapolation from one concentration-duration to another. (This abstract does not necessarily reflect EPA policy.)

**MODELING THE IMPACT OF WORKLOAD ON THE BIOLOGICAL EXPOSURE INDICATORS OF STYRENE: COMPARISON BETWEEN SINGLE EXPOSURE AND BINARY EXPOSURE WITH ACETONE.**

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Toluene, a solvent used in numerous consumer and industrial applications, exerts its critical effects on the brain and nervous system following inhalation exposure. Our previously published PBPK model successfully predicted toluene concentrations in blood and brain over a range of conditions, but in recent experimental studies it over-predicted (by ~2-fold) concentrations in blood and brain after 24 hrs of continuous exposure to 775 or 1125 ppm toluene. The goal of this current modeling effort was to determine if changes in physical activity patterns (as measured by telemetry) and/or enzyme induction could explain toluene pharmacokinetics following 24 hrs of continuous exposure to toluene. Compartmental models in the model are lung, slowly and rapidly-perfused tissue groups, fat, liver, GI tract and brain; tissue transport is blood-flow limited and metabolism occurs in the liver. Chemical-specific parameters and initial organ volumes and blood flow rates were obtained from the literature. Observed changes in motor activity and heart rate during the period of exposure were implemented in the model by increasing cardiac output and alveolar ventilation up to 10%, but this was insufficient to account for the observed pharmacokinetic behavior. Alternatively, incorporation of cytochrome P450-mediated enzyme induction (increasing VmaxC up to 5-fold) allowed successful prediction of the experimental data. The degree of enzyme induction tested was biologically plausible based on literature data for shorter term toluene exposure which report up to 8.3-fold induction in CYP2E1 activity. Our previously published model predicted total toluene concentrations in blood and brain following 24 hrs of exposure to 500 ppm toluene (ACGIH) with a 5.3-fold difference in predicted and measured concentrations for the model with no enzyme induction including 2-fold differences in brain concentrations. This highlights the potential impact of enzyme induction when simulating chronic toxicity studies for purposes of risk analysis, i.e. when using a PBPK model to obtain estimates of internal dose for application to dose-response analysis. (This abstract does not necessarily reflect EPA policy.)
APPLICATION OF TISSUE-TIME COURSE DATA TO ELUCIDATE MECHANISTIC DETAILS OF CARBON TETRACHLORIDE (CCL4) TRANSPORT USING AN UPDATED PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL IN RATS.

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CCL4 is a common environmental contaminant in water and superfund sites, and a model liver toxicant. One application of PBPK models used in risk assessment is simulation of internal dose for the metric involved with toxicity, particularly for different routes of exposure. Time-course pharmacokinetic data for different tissues (Sanzgiri et al., 1997) were used to evaluate a rat PBPK model employing previous metabolic estimates. The flow-limited PBPK model contained: fat, liver, brain, rapidly and slowly perfused compartments. Arterial concentration data measured for 100 or 1000 ppm constant inhalation exposure lasting 2 hours were used to evaluate the initial PBPK predictions. These simulations were able to describe the time-course data well at both inhaled concentrations. However, a closer examination of tissue uptake data of 1000 ppm CCL4 revealed an inconsistency with the preliminary simulations. Upon further evaluation of tissue-time course results, the PBPK model underpredicted brain and fat tissue concentrations. Also, the peak arterial concentration was about twice that predicted by the initial calibration simulation. Further modeling is needed to incorporate physiological and structural details (i.e. diffusion) that may be taking place at higher exposure concentrations. Ongoing model refinement strategies will include: (1) addition of a blood brain barrier component (for brain tissue), and (2) a diffusion-limited compartment for fat, and (3) diffusion in the respiratory tract during the breathing cycle. Diffusion may help explain the difference between observed and predicted tissue concentrations. In summary, tissue-time course data are essential for the determination of mechanistic detail for different organs, and the accurate prediction of internal liver dose. (This abstract does not reflect EPA policy.)

ALTERNATIVE APPROACH TO MAXIMUM FLUX FOR TTC APPLIED TO SAFETY EVALUATION OF COSMETIC INGREDIENTS.


Relevant and accurate prediction of dermal uptake/exposure of topically applied chemicals is essential for risk assessment. It could be obtained without recourse to an experimental measurement and avoids any problems with ethical issues, recruiting volunteers or housing animals. Typically, QSAR model predicting permeability coefficients (i.e. kp) are used. Many models were developed; all of them lead to the same conclusion: small lipophilic chemicals have greatest skin permeability. This analysis often rises to confusion. Dataset used to build up this relation concerns percutaneous transport from aqueous solution. Whereas, kp increases with log P , aqueous solubility decreases with lipophilicity. Resulting flux, and effective absorbed amount of chemical, is then balanced between two competitive factors (permeability and solubility).

Concept of maximum flux means that a chemical cannot cross the skin higher than flux measured at steady state with a chemical applied on the surface in saturated solution (or in neat chemical form). It allows assessing the maximum absorbed dose. This concept was recently used in TTC approach for cosmetic ingredient. A classification of potential of cutaneous chemical absorption was proposed on the basis of their physico-chemical properties. Unfortunately, the proposed classification does not cover all range of molecular weight and log P . Moreover, the default proposed values greatly overestimate the absorption experimentally obtained.

To overcome these limitations, a QSAR model recently developed by L’Oreal can be used. It estimates the cumulative mass of a chemical absorbed into and through the skin in typical ‘in-use’ cosmetic conditions. Applicability domain is clearly defined and covers a wide range of physico-chemical properties. Moreover, the model was build up with 101 data. More than 90% were well predicted (i.e. difference between predicted and experimental values less than a factor 5). At least, improvement was recently done to take into account property of volatile chemicals.
UNVEILING ASSOCIATIONS BETWEEN LACTATIONAL EXPOSURE TO POLYCHLORINATED DIPHENYLS (PCBs) AND INFANT NEURODEVELOPMENT: USE OF PBPK MODELING VS TRADITIONAL EXPOSURE METRICS.

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Traditional approaches to assess lactational exposure to PCBs in epidemiologic studies rely on levels measured in biologic specimens. Because of the multiple concurrent toxicokinetic processes involved in postnatal exposure, such metrics are unlikely to embody the complete infant internal exposure profile. We conducted this study to compare exposure estimates generated by different approaches and their sensitivity in screening for associations with neurodevelopmental outcomes in northern Quebec Inuit infants. Postnatal exposure was calculated using published metrics based on samples of breast milk (1 month postpartum) and infant blood (at 6 months of age) and through PBPK modeling of mother-infant lactational transfer. PCB-153 was used as a proxy of exposure to a mixture of PCBs. As expected, breast milk levels were found to be more highly correlated with cord blood levels than with infant blood levels at 6 months and are, therefore, surrogates of prenatal exposure. When breast milk levels were multiplied by the duration of breast-feeding, estimates were highly correlated with infant blood levels (Pearson r = 0.78). However, PBPK-simulated concentrations were even better predictors of infant levels (Pearson r = 0.86). Using PBPK-simulated monthly area under the curve, associations were found between PCB-153 levels during specific periods and two neurodevelopmental outcomes (Pearson r = 0.86). Using PBPK-simulated monthly area under the curve, associations were found between PCB-153 levels during specific periods and two neurodevelopmental outcomes (Pearson r = 0.86). Using PBPK-simulated monthly area under the curve, associations were found between PCB-153 levels during specific periods and two neurodevelopmental outcomes (Pearson r = 0.86).

There is a growing interest in using in vitro data and pharmacokinetic (PK) modeling to improve risk assessments of chemicals in humans. Carbamyl is a widely used anti-cholinesterase (ChE) insecticide that has been linked to significant human exposure. In the present study, parameters for metabolism and ChE inhibition of carbaryl were determined in vitro to refine the previously developed rat pharmacologically based pharmacokinetic (PBPK) model. Metabolism was determined in freshly isolated hepatocytes from adult male Sprague Dawley rats. Carbaryl disappearance followed Michaelis-Menten kinetics with Vmax of 1.3 nmol/min/10^6 cells and apparent KM of 50 - 80 μM. Interactions between carbaryl and ChEs were determined in brain, red blood cells (RBCs), and plasma. Bimolecular inhibition rate constants (Ki) were 2 - 12 μM^-1 h^-1 for acetylcholinesterase (AChE) suggesting differing degrees of sensitivity of AChEs in RBCs, brain, and plasma. The Ki for butyrylcholinesterase (BChE) in plasma was lower than AChE. The rates of regeneration of the carboxylated ChEs were 0.5 - 2 h^-1 indicating similar rates of recovery for AChE and BChE. These in vitro PK and pharmacodynamic data were extrapolated to whole animal to refine the description of the metabolism and ChE inhibition dynamics of carbaryl in the model. Predicted tissue carbaryl concentrations and ChE inhibition profiles in brain and blood were in better agreement with the observed data compared to the previous modeling, in which the metabolic and ChE inhibition parameters were all estimated from in vivo kinetic data. The refined rat model will be scaled up to a human model with data from in vitro studies using human tissues. The ultimate goal is to demonstrate a process for developing a human PBPK model for risk assessment based on an animal PBPK model augmented with in vitro to in vivo extrapolation approaches using human tissues. The resulting model will be used as a template for developing models for other N-methyl carbamates to support a cumulative risk assessment and interpret biomonitoring data on this class of pesticides.

A biologically based dose response (BBDR) model for the lactating rat and pup hypothalamic-pituitary-thyroid (HPT) axis is being developed to advance understanding of the thyroid hormone disruptions and neurodevelopmental neuropsychiatric (DNTP) impairments caused by inadequate dietary iodide (ID). In our lab, the model for the lactating rat and pup quantify the compensatory mechanisms that govern the relationships between serum and thyroid hormone levels (e.g., TSH). Brain T3 and T4 concentrations in the lactating dam and the nursing neonate, recognizing that these relationships may be affected by the mechanism of toxicity of different compounds. Initially, the model will be used to delineate perturbations in the HPT axis caused by inadequate dietary iodide (ID). Later, environmental toxicants that alter HPT axis homeostasis will be examined. The current model uses the McLanahan et al. (2009) BBDR-HPT axis model for the adult rat as a foundation, but includes several new features: 1) formation rates of THs, 2) negative feedback loop controlled by model-predicted brain concentrations of T3, 3) extrathyroidal metabolism of THs by deiodinase enzymes, 4) regulation of deiodinase II in the brain, 5) serum protein binding of THs, and 6) maternal excretion of TSH and iodide via the milk. Algebric equations were developed to describe physiological changes for the dam and nursing pups. The model is calibrated to predict perturbations in the HPT axis caused by ID and to ascertain the pup HPT axis tolerance to maternal ID during the nursing period. (Support: U.S. EPA STAR Cooperative Agreement R832134 and AFRL through the Henry Jackson Foundation for the Advancement of Military Medicine Contract 185137. This abstract does not necessarily reflect EPA policy.)
897 DEVELOPMENT AND DEMONSTRATION OF A COMPUTATIONAL FRAMEWORK FOR FORWARD AND REVERSE DOSIMETRY OF ORGANOPHOSPHORUS INSECTICIDE MIXTURES.
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Although various biomarkers have been used to assess exposure to and poisoning from organophosphorus (OP) insecticides, the complexity of OP absorption distribution, metabolism, and elimination warrants integration of computer-assisted modeling tools with the biomarker data for more accurate quantitation and assessment of target tissue dosimetry. Here we describe the development and current capabilities of a novel computational framework for dosimetry of OP insecticide mixtures, DoseSim:OP. This framework has features for both forward dosimetry, in which a calibrated model is used to predict biomarker data from known exposures, and reverse dosimetry, in which a calibrated model is used to reconstruct dose and exposure from collected biomarker data. To this end, DoseSim:OP implements a graphical user interface to tools for Monte Carlo and Bayesian analyses, statistical post-processing and visualization of results. As a first step toward a general OP mixture dosimetry model, we have implemented a physiologically-based pharmacokinetic (PBPK) model for a mixture of chlorpyrifos [O,O-dimethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] and diazinon [O,O-dimethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl)-phosphorothioate], including both specific and non-specific metabolites. Using this model in DoseSim:OP, we found good agreement between reconstructed and experimental dosing scenarios using in vivo biomarker data for rodents as input. Future developments of DoseSim:OP will include additional validation from targeted in vivo studies, validation using human exposure data, and dose reconstruction using biomarker levels from human epidemiological databases. This project was supported by EPA STAR Grant R833451.

898 A SYSTEMS MODEL OF BILE SALT METABOLISM AND ITS APPLICATIONS TO CHOLESTASIS.
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Predicting the cholestatic potential of drugs is difficult since most drugs affect multiple cellular mechanisms making the combined impact difficult to forecast. Genetic polymorphisms only add to the complexity of making individual level predictions. The multi-factorial origin of cholestasis, each factor not necessarily capable of causing cholestasis by itself, raises the difficult question of what combination of factors does cause cholestasis, and to what extent. The ability to answer such questions provides insights into who develops what complications on exposure to a particular drug or drug combination and how helping in individualized therapy. To address this issue, we have developed a dynamic systems model of bile formation and metabolism that simulates such complex scenarios and provides insights into cholestatic disease at an individual level. To illustrate the principle, we input the data for rodents as input. Future developments of DoseSim:OP will include additional validation from targeted in vivo studies, validation using human exposure data, and dose reconstruction using biomarker levels from human epidemiological databases. This project was supported by EPA STAR Grant R833451.

899 A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELE FOR PRAILDIXOME IN THE GUINEA PIG AND HUMAN.
Chemical warfare agents such as organophosphorus compounds inhibit hydrolysis of acetylcholinesterase (AChE) at the synaptic junctions by phosphorylation of ester group. This will result in accumulation of acetylcholine in the synapses, causing seizures to muscle paralysis, and even death due to respiratory failure. To prevent overstimulation of acetylcholine at the synapses, AChE breaks down acetylcholine into inactive forms. Pralidoxime (2-PAM) reactivates AChE by removing the phosphoryl group bound to the ester moiety of the enzyme. The objective of this work was to provide quantitative dose-response relationships of Pralidoxime (2-PAM) across multiple species and multiple routes of chemical warfare nerve agent (CWNA) exposures using a physiologically based pharmacokinetic model. The model developed in this study accurately simulated blood concentrations of 2-PAM in the guinea pig and human. Quantitative structure-activity relationship (QSAR) algorithms were used to develop PBPK model parameters for 2-PAM, based on the basic physicochemical properties and the octanol-water partition coefficient data collected. Predicted tissue/blood partition coefficients of 2-PAM in guinea pig and human were used as preliminary values to simulate the model. Data from the literature were used to predict blood time course in the guinea pig after intramuscular injection of 2-PAM (3.5 and 25 mg/kg). The model predictions of blood kinetic data were in good agreement with experimental values. Human blood kinetic data was also simulated with this model using human physiological parameters. There was a good agreement between a model simulation and experimental data from injection of 5 mg/kg 2-PAM intravenously. Oral uptake in human will be included in this investigation. A PBPK model developed in this study adequately predicted blood kinetics of 2-PAM in guinea pig and human. This study was supported by the Defense Threat Reduction Agency.

900 ADAPTIVE RESPONSES TO PROCHLORAZ EXPOSURE IN THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS OF FATHEAD MINNOWS.
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Exposure to endocrine disrupting chemicals can affect reproduction and development in both humans and wildlife. We are developing a mechanistic mathematical model of the hypothalamic-pituitary-gonadal (HPG) axis in female fathead minnows to predict dose-response and time-course (DRTC) behaviors for endocrine effects of the fungicide prochloraz. The model includes several feedback regulatory loops within the HPG axis that mediate adaptive responses to endocrine stress. Fathead minnows were exposed to prochloraz at 30 or 300 μg/L for 8 days followed by an 8-day recovery phase. Adaptive changes in plasma estradiol levels occurred during exposure and treatment-related oscillations occurred post-exposure. Computer simulations were performed to compare the model-predicted DRTC experimental and data to gain insight into how the feedback control mechanisms embedded in the HPG axis mediate these changes. As this work progresses we will obtain a refined understanding of how adaptive responses within the HPG axis of fathead minnows affect DRTC behaviors for prochloraz. This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect Agency policy. M. Brenn was supported by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT833235-01-0 with North Carolina State University.

901 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR TRIADIMEFON AND TRIADIENMOL IN RATS AND HUMANS.
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A physiologically based pharmacokinetic (PBPK) model was developed for the conazole fungicide triadimefon and its primary metabolite, triadimenol. Rat tissue blood partition coefficients and metabolic constants were measured in vitro for both compounds. Kinetic time course data for parent and metabolite were collected from dose dependent cellular uptake at the basal-lateral membrane, ATP dependent transport at the canalicular membrane and the membrane fluidity often observed in higher doses of ethinyl estradiol. We overlaid pharmacokinetics to this picture. For example, we applied the trans-inhibitory effect of estradiol-17 beta-glucuronide, a metabolite of estrone on BSEP after its excretion by MRF2 into the canalicular lumen. To account for individual variations in response, we studied the effect of mutations at highly conserved loci such as D676Y and G855R in BSEP that are associated with drug induced liver injury and V444A implicated for predisposition to estrogen mediated cholestasis. The model predictions of the advent and the extent of cholestatic disease were well in concordance with experimental observations. In addition the model clarified the role of each of the above mentioned mechanisms in disease. We thus illustrated the application of a dynamical systems approach which allows the study of complex biological factors responsible for cholestasis and allows one to understand mechanisms and treatments.
using area under the concentration curve (AUC) in brain and blood for triadimefon and triadimenol as dosimetrics. All dosimetric-based HEDs were above the oral reference dose of 0.11 μmol triadimefon/kg/day.

902 A DERMAL ABSORPTION MODEL BASED ON SUCCESSIVE PARTITIONING THROUGH A NON-HOMOGENOUS STRATUM CORNEUM LIPID MATRIX.

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Models of dermal absorption typically treat the stratum corneum lipids as a homogenous medium through which solutes diffuse according to Fick's first law of diffusion. This approach does not explain non-first order diffusion observed experimentally when the dose rate varies. Successive partitioning through a non-homogenous stratum corneum lipid matrix offers an alternative approach that can simulate non-linear dermal absorption patterns. It is based on a conceptual model of the stratum corneum that includes varied local physical-chemical characteristics and dynamic solute effects within the lipid matrix. In this approach the rate of solute exchange between distinct lipid domains become rate limiting, rather than the rate of diffusion through a relatively voluminous, homogenous medium. The approach is demonstrated using a large, sparse and regular network model where nodes have variable characteristics, including limitations on node capacities for solutes that simulate variable solubility limits in different regions within the stratum corneum lipid matrix. Rates of solute movement from node to node is determined by the rates of solute movement within nodes, as characterized by diffusivity, and the rates of exchange between nodes, as characterized by partitioning coefficients. The network outputs production absorption to dose relationships that can be characterized using power equations, similar to equations used to describe absorption to dose relationships in experimental dermal absorption data.

903 THE UTILITY OF THE MINI-PIG IN P38 MAP KINASE INHIBITOR TESTING.

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P38 map kinase inhibitors are investigated as a target for many types of inflammatory diseases due to their inhibitory action on pro-inflammatory cytokines such as TNFα and IL-1β and other inflammatory mediators. The repeat dose toxicity of a P38 MAPK inhibitor was evaluated in rats and minipigs. The minipig was chosen as the non-rodent species as there were no major qualitative or quantitative differences between man and minipig in an in vitro metabolic profiling study. Toxicity included lymphoid proliferation in rats and GI inflammation in minipigs characterised by increased WBC and acute phase reactants and confirmed histologically. A number of target related toxicities have previously been reported clinically and preclinically e.g. hepatic, cardiac, CNS and skin toxicities but were not observed in these studies. Rats were much less sensitive towards the adverse effects of the compound and tolerated doses 10-100-fold higher than minipigs although the reason for the difference in sensitivity is not known. Previously Davis (SOT 2008) reported that species specific toxicities have been observed with kinase inhibitors and the dog is uniquely sensitive to p38 MAPK toxicity due to over expression of p38 in B lymphocytes leading to acute lymphoid necrosis and colonic haemorrhage: thus the non-human primate (NHP) has traditionally been selected as the second species. Despite the minipig being more sensitive than the rat, it was considered to be much less sensitive than the dog and comparable to the NHP and human. These data demonstrate that the minipig is an excellent alternative to the primate for the toxicological evaluation of p38 MAPK inhibitors and thus in line with the directive on animal experimentation, 86/609/EEC aimed at reducing the number of NHP. The minipig does not share the problems encountered with the use of the dog due to over-expression of p38 and demonstrated excellent concordance with the human. Also, the minipig was shown to be a reasonable predictor of human AUCinf and Cmax concentrations.

904 ZINC FINGER NUCLEASE MEDIATED CREATION OF RODENT KNOCKOUT MODELS ON TOXICOLOGY.

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Animal models are an essential element of drug development and toxicity assessment. Rats are preferred over mice for their closer resemblance to human anatomy and physiology. Additionally, their larger size allows for multiple sample collections in pharmacokinetics studies. Until now, knockout rats were not readily available due to limitations in embryonic stem cell technology. Here we report on the creation of a suite of rat knockouts on toxicology targets and the first knockout mouse (Mdr1a -/-) generated using the zinc finger nuclease (ZFN) technology. ZFNs are injected into one-cell rodent embryos to introduce site-specific modifications on the chromosome, leading to the disruption of gene function (1). Similar to creating a transgene, knockout founders can be obtained and analyzed at a high rate in 6-8 weeks of time from the time of injection. For example, 77% of live births from mouse embryos injected with Mdr1a ZFNs were founders, carrying deletions ranging from 7 bp to 695 bp around the target site, whereas for Mdr1a knockout rats, 12.5% live births were founders with deletion of 6 bp - 21 bp. In addition to Mdr1a, we are in the process of creating rats with Bcrp, PXR, Mrp1 and Mrp2 disrupted, respectively. Detailed genotypes and injection statistics of each model will be reported. Homozygous animals will be phenotyped. Furthermore, we have recently generated a p38 knockout rat model that has potential applications in genotoxicity. References: 1. Geurts et al (2009). Knockout rats via embryo microinjection of Zinc-finger nucleases. Science. Vol. 325, 433.

905 GSH-DEPLETED ERYTHROCYTE RAT MODEL OF DRUG-INDUCED HEMOLYTIC ANEMIA.

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We describe studies to develop a primaquine-sensitive hemolytic anemia animal model using male Sprague-Dawley rats. To increase the sensitivity of normal rat erythrocytes to pro-oxidant hemolytic agents, donor rat red blood cells (RBCs) were incubated and treated in vitro with a sufficient amount (2.65 mM) of diethyl maleate (DEM) to deplete GSH by >95%. GSH-normal and GSH-depleted RBCs were stained with the fluorescent cell-tracking dyes DiD and DiO, respectively, mixed together in an equal ratio, and returned to the circulation of syngeneic recipient rats. Experiments were performed with this rat model using dapson (0-120 mg/kg) and primquine (0-50 mg/kg/day x 5 days). Treatment of the rats with dapson provoked a dose-dependent increase in the rate of removal of the dye-tagged RBCs, and decreased the ratio of DiO:DiR RBCs, indicating that the GSH-depleted RBCs were removed preferentially over the GSH-normal RBCs. In contrast, primquine treatment did not affect RBC survival as determined by lack of detectable change in the GSH-depleted RBC/GSH-normal RBC ratio. However, 75% of the rats in the primaque high dose group (50 mg/kg/day) died during the course of the experiment. Overall, the data support the proof of concept; however, rats may not be the optimal species to use in pursuit of this animal model. This research is supported by a sub-contract (08-04-072) from the University of Mississippi (USAMRMC Award # W81XWH-07-2-0095).

906 EVALUATION OF SCORE METHODS FOR THE PREDICTION OF DRUG-INDUCED LIVER INJURY IN HUMANS BY USING CHIMERIC PXB-MICE® WITH HIGHLY HUMANIZED LIVER.

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Of the leading causes of withdrawal of a newly developed drug, drug-induced liver injury (DILI) takes a significant position. Toxicogenomic approaches have been implemented using in vivo (experimental animals, mainly rats) or in vitro (human hepatocytes or hepatoma cells) systems in order to find biomarkers for DILI. We have used chimeric PXB-mice®, in which more than 70% of hepatic parenchymal cells are replaced by human hepatocytes, for the toxicogenomic analyses of hepatocarcinogens. This animal model, which mimics human-type drug metabolism, has a potential to bridge the gap between rodent-type and human-type liver and explain the difference of in vivo and in vitro response of human hepatocytes against hepatocarcinogens. Using different 20 hepatocarcinogens (acetaminophen, amiodarone, diclofenac, d-penicillamine, ethromycin, valproate, sulindac, indomethacin, perhexilene, methylthyla, amitriptyline, tamoxifen, acetylsalicylic acid, methotrexate, demeclocycline, hydrazine, hydroxyurea, imipramine, orotic acid) and seven non-hepatotoxicants, we have analyzed changes in hepatic gene expression in rats and PXB-mice®. These drugs were orally administered to rats and PXB-mice® three-times daily at high doses (ca. 20% of reported LD50), followed by hepatic total RNA preparation and gene expression analyses using oligonucleotide microarray chips. Several maker gene candidates, which specifically
responded to the hepatotoxins, were extracted and evaluation of DILI susceptibility due to the drug-treatment was analyzed by using two different score methods. Lot-to-lot difference of chimeric mice on the effectiveness of score methods will be discussed.

907 DIFFERENTIAL EXPRESSION OF PROTEINS IN THE LIVERS OF HEPATIC ALCOHOL DEHYDROGENASE-DEFICIENT DEER MICE AFTER SUBCHRONIC EXPOSURE TO ETHANOL.
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In an earlier study, we reported a significant fatty degeneration (steatosis) and injury in the livers of hepatic alcohol dehydrogenase (ADH)-deficient (ADH-) vs. hepatic ADH-normal (ADH+) deer mice fed ethanol. Steatosis is a known potential risk factor for initiation and progression of alcoholic liver disease (ALD) suggesting that proteins involved in lipid metabolism and transport are the target of ethanol toxicity in ADH-deer mice. Therefore, we evaluated differential expression of proteins involved in lipid metabolism and transport are the target of ethanol toxicity in ADH-deer mice. We used MALDI-TOF mass spectrometry of the differentially expressed proteins. A total of 23 proteins in ADH-deer mice fed ethanol vs. ADH-pair-fed controls and 9 in ADH+ deer mice in the livers of ADH- and ADH+ deer mice fed 4% ethanol via liquid diet daily for 90 days. Therefore, we evaluated differential expression of proteins involved in lipid metabolism and transport are the target of ethanol toxicology in ADH-deer mice. We used MALDI-TOF mass spectrometry of the differentially expressed proteins. A total of 23 proteins in ADH-deer mice fed ethanol vs. ADH-pair-fed controls and 9 in ADH+ deer mice fed ethanol vs. ADH+ pair-fed controls were differentially expressed. Heme-, fatty acid- and copper binding proteins, annexin A4, heat shock protein 8 and proteins involved in fatty acid β oxidation (acyl-coenzyme A dehydrogenase and enoyl coenzyme A hydratase) were differentially expressed only in ADH-deer mice fed ethanol vs. ADH-pair-fed controls. However, similar proteins were differentially not expressed in the livers of ADH+ deer mice fed ethanol. These findings suggest that metabolism of ethanol under hepatic ADH deficiency (a metabolic condition found during chronic alcohol abuse) plays an important role in fatty degeneration, apoptosis, and activation of endoplasmic reticulum stress in the early stage of ALD.

908 DETERMINATION OF TETRABROMOBISPHENOL A IN RAT SERUM AND URINE BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY.
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Tetrabromobisphenol A (TBBPA), one of the most widely used brominated flame retardants in the world, is used to improve fire safety of laminates in electrical and electronic equipment. A liquid chromatography-tandem mass spectrometry was developed for the determination of TBBPA in rat serum and urine, and applied to the toxicokinetic study of TBBPA in rats. Acceptable linearity was observed over the concentration ranges of 0.25 - 50 and 0.01 - 5 μg/ml in serum and urine, respectively. The limits of detection of TBBPA were 0.04 μg/ml in serum, and 0.0025 μg/ml in urine. The precisions for the assay in serum and urine were below 13 and 14%, respectively, and the accuracies ranged 95 - 111% and 98 - 101% for intra-day and 97 - 107% and 97 - 102% for inter-day, respectively. Serum and urine concentrations of TBBPA were successfully monitored following oral administrations at the dose of 200 mg/kg rats. The present results suggested that the method developed affords the sensitivity, accuracy and precision necessary for quantitative measurements in toxicokinetic studies of TBBPA in vivo.

909 ENHANCED EXPRESSION AND ACTIVITIES OF MMP-9 AND NADPH OXIDASE IN CENTRAL NERVOUS SYSTEM AND SPLEEN TISSUES OF EAE MICE MODEL.
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Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease of demyelination and axonal damage that share many features with multiple sclerosis (MS). NADPH oxidase is an important source of reactive oxygen species in different brain regions and in different cells including neurons. The objective of this work is to investigate the association between matrix metalloproteinase-9 (MMP-9) and NADPH oxidase activities in EAE mice model. EAE was induced by subcutaneous injections of MOG35-55 emulsified in IFA containing H37Ra Mycobacterium tuberculosis. Pertussis toxin was injected intraperitoneally on days 0 and 2 after immunization. From day 10 body weight was decreased and clinical score was increased gradually in EAE mice. MMP-9 activity was measured by western blot and zymography in brain, spinal cord, spleen, and plasma samples. In brain, spinal cord, and spleen MMP-9 levels were increased by 21, 59 and 23% by western blot and by about 7, 11 and 12 % by zymography, respectively. Plasma samples showed an increase in MMP-9 levels at days 10, 21 and 30 of EAE mice (36, 89 and 65 % by western blot; 10, 28 and 13 % by zymography, respectively). Specific NADPH oxidase activity was increased in brain, spinal cord, and spleen of EAE mice compared to control by luminescence spectrometer. It showed that NADPH oxidase activity in plasma samples was increased at day 10, 21 and 30 of EAE mice. Total NADPH activity at days 10, 21 and 30 was increased by 8, 55 and 44 %, respectively. Specific NADPH oxidase activity at days 10, 21 and 30 was increased by 13, 13 and 7 %, respectively. Specific SOD activity at days 10, 21 and 30 was increased by about 8, 11 and 10 %, respectively. Plasma MMP-9 and NADPH oxidase activities can be a useful biomarker for predicting EAE disease. MMP-9 was in part correlated with NADPH oxidase activity in plasma.

910 ESTABLISHMENT OF 2-STAGE SKIN CARCINOGENESIS MODEL IN CB6F1 TG RASH2 MICE.
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Short-term carcinogenicity studies using rasH2 mice have recently been performed in place of the conventional 2-year carcinogenicity study. In this study, we tried to establish an ultra-short-term carcinogenicity study in which the target was the skin, one of the common targets in rasH2 mice. [Exp. 1 DMBA as initiator] 50, 10, 2 or 0 μg DMBA was applied to shaved dorsal skin of male and female rasH2 mice (5 mice in each group). A skin tumor in the applied area was observed at 50 μg in a male at 19 weeks after DMBA treatment and at 2 μg in a female at 20 weeks after DMBA treatment. By 26 weeks after DMBA treatment when all mice were sacrificed, skin tumors in the applied area were observed at 10 μg in one male and female, and at 50 μg in one female. Tumor incidences were not statistically different among sex or concentrations of DMBA. [Exp. 2 TPA as promoter] 8 μg TPA was applied to shaved dorsal skin of female rasH2 mice twice a week from one week after 50 μg DMBA application. At 4 weeks after DMBA treatment, skin nodules were observed in the DMBA+TPA treated group, in which the incidence reached 100% in the following week. At 8 weeks after DMBA treatment, all mice underwent necropsy because many skin nodules were observed. Incidence / multiplicity of skin tumors was 0% / 0% in the DMBA treated group, 100% /62.4 in the DMBA+TPA treated group and 20% /0.2 in the TPA treated group. [Conclusion] It was confirmed that latent periods of skin tumor induction after 50 μg DMBA + 8 μg TPA treatment in rasH2 mice were very short, less than half of those in ICR mice.

911 CARCINOGENIC SUSCEPTIBILITY MONITORING OF CB6F1 TG RASH2 MICE IN THE EARLY STAGE OF A SHORT-TERM CARCINOGENICITY TEST.

High carcinogenic sensitivity and reproducibility for human carcinogens in rasH2 mice have been validated in a large number of certification studies (Yamamoto et al., 1998; Usui et al., 2001). Although it had been recommended to use a positive control group in every short-term test to qualify the carcinogenic susceptibility of the mice, the necessity of a positive control group for every test was discussed at annual meeting of Society of Toxicologic Pathology 2009. The positive control group is usually treated with N-methyl-N-nitrosourea (MNU), and tumor incidences by 26 weeks after MNU administration are monitored to qualify the carcinogenic susceptibility of corresponding rasH2 mice. Since we found that the forestomach was the most sensitive organ to MNU administration (SOT in 2008), we investigated if the incidences of forestomach papilloma would be useful as an index of carcinogenic susceptibility in the early stage of a short-term carcinogenicity test. In this study, the incidences and the multiplicities of forestomach papillomas in rasH2 mice at 13 weeks after MNU administration were 100% in both genders. Mean numbers of forestomach papillomas in rasH2 mice at 13 weeks and 26 weeks after MNU administration (75 mg/kg, single intraperitoneal injection) were investigated. Furthermore, the incidences of tumors other than forestomach papillomas at both time points were compared as background data. The incidences of forestomach papillomas at 13 weeks after MNU administration were 100% in both genders. Mean numbers of forestomach papillomas at that time were 10.6 in males and 12.3 in females. Since there was no difference between tumor incidences at 26 weeks after MNU administration and background data, the incidence of forestomach papilloma at 13 weeks after MNU administration seemed to be useful as an index to monitor carcinogenic susceptibility of rasH2 mice.
COMMERCIAL FISH DIETS INDUCE VITELLOGENIN PRODUCTION IN MALE TILAPIA (OREOCROMIS MOSSAMICUS), A CONSIDERATION FOR ENDOCRINE DISRUPTOR STUDIES.

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In a number of fish species, male plasma has been shown to contain substantial levels of 17ß-estradiol (E2) and in certain cases, vitellogenin (Vg), an E2-induced precursor of egg yolk protein that is specific to females. The presence of E2 and Vg in males raises questions about their etiology and may confound the assessment of environmental endocrine disruptors in aquatic species. In order to examine whether components of commercial fish feed might induce Vg production, Vg levels were measured in male tilapia fed either commercial feed, commercial feed treated with E2 (20 mg/kg of feed), or a diet consisting of 50% squid and 50% mixed vegetables. Over 20 days, plasma Vg of fish fed commercial feed remained constant at 3-7 mg/ml while levels of fish fed E2 significantly increased to 45 mg/ml. Plasma Vg of squid/vegetable-fed fish declined over time to often undetectable levels. Expression of three Vg genes (Vgs A-C) in the liver of squid/vegetable-fed fish decreased to 0.6-2.2% of levels in control fish after 20 days while expression of Vgs A-C in E2-fed fish increased by up to 25 fold. Custom made fishmeal- and soy-based diets were also tested for Vg induction potential. While plasma Vg was reduced after 20 days in male fish fed the soy-based diet, plasma levels were still detectable. The data suggest that, most likely from the fish meal component of the feed, induced gene expression and production of Vg in male tilapia. Sustained use of this diet will maintain Vg production, and may mask effects in studies of estrogenic endocrine expression and production of Vg in male tilapia. Sustained use of this diet will assess the effects of endocrine disruptors in fish.

ANIMAL MODEL OF AUTISM USING GSTM1 KNOCKOUT MICE AND EARLY POSTNATAL VPA TREATMENT.

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The etiology of autism is thought to involve a gene by environmental insult by age of exposure interaction. To test this hypothesis, mouse pups with a deletion of glutathione-S-transferase M1 (a gene associated with increased risk of autism and that codes for an enzyme involved in the management of toxicant-induced oxidative stress) and wild-type controls were exposed to valproic acid (a toxicant known to cause autism-like behavior deficits following prenatal exposure and one that exerts its toxicity, in part, by inducing oxidative stress) on post-natal day 14 (P14). These pups were then a) sacrificed 24 hours following injection and whole brains were taken for TUNEL staining in order to detect the presence of apoptotic cells; or b) allowed to mature until P30 and then observed for social, non-social and motor behavior maturation; or c) allowed to mature until P30 and sacrificed for neurochemistry analysis of monoamines and their metabolites in four different brain regions (cerebellum, hippocampus, striatum and frontal cortex). It was observed that VPA-treatment resulted in significant increases in the number of granule cells staining for TUNEL in the hippocampus and cerebellum. There was also a gene by treatment by sex interaction with VPA-treated wild-type females having increased protection against VPA-induced cell death. VPA-treatment also resulted in long-lasting deficits in social behaviors and corresponding changes in brain chemistry. In addition to treatment effects, there were genotype effects in both social behavior and neurochemistry between the saline treated GSTM1 knockout and wild-type pups. Collectively, these data expand our current animal model of autism by adding a genetic component in the form of an autism susceptibility gene. In addition, these results support the hypothesis that autism may be the result of a gene by toxicant interaction wherein both factors share a common feature of oxidative stress.

DEVELOPMENTAL TOXICITY STUDY OF CBLB502 IN WISTAR RATS.

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CBLB502 is a derivative of a microbial protein, which has demonstrated the capacity to reduce injury from acute stresses, such as radiation, in animal models. CBLB502 is an activator of nuclear factor kappa B (NF-KB) and is a specific Toll-like receptor 5 (TLR5) agonist. The objective of the study was to determine the potential developmental toxicity of CBLB502 in Wistar rats. Four groups of 25 time-mated female Wistar (Crl:WI) rats/group received subcutaneously 0, 30, 100, and 300 μg/kg/day of CBLB502 from Gestation Days (GD) 6 to 17, at a dose volume of 1.0 mL/kg. Additionally, one group of three animals and three groups of nine animals/group served as TK animals and received the vehicle or CBLB502 in the same manner as the main study groups. On GD 20, uterine examination was performed in which the total number of implantations, early and late resorptions, viable and nonviable fetuses, sex, and individual fetal weights were recorded. Fetuses were examined for external, internal, skeletal, and visceral abnormalities. No CBLB502-related malformations were observed during the study. Gestation body weight and gestation body weight changes were adversely affected at 100 and 300 μg/kg/day. Gestation food consumption was significantly reduced in all treated groups; however, the reduction in gestation food consumption at 30 μg/kg/day was not considered to be adverse. No external malformations and CBLB502-related visceral malformations/variantions were observed. The incidence of incomplete ossifications of parietal, squamosal and supra occipital bones were slightly increased at 100 and 300 μg/kg/day, but not statistically significant. Delayed ossification is often related to maternal toxicity and is probably not predictive of teratogenic potential. Based on the results of this study, the NOAEL (No Observed Adverse Effect Levels) for maternal and developmental toxicity were determined to be 30 and 100 μg/kg/day, respectively. Preclinical safety results available to date support the continued development of CBLB502 in man.

SCHEDULED SAMPLING OF CARDIOVASCULAR PARAMETERS: HOW OFTEN SHOULD ONE COLLECT DATA.

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The growth of the aging population highlights the need for laboratory animal models that can be used to (1) efficiently monitor the health of aging research colonies, and (2) aid in unraveling the mechanisms of susceptibility to toxic chemicals and disease. An observational assessment was therefore developed that provides rank scores of appearance, motor activity, and muscle tone on a 5-point scale. A score of 1 indicated no impairment while a score of 5 indicated severe impairment. The assessment of a single rat can be completed in about one minute. The assessment was applied to male Brown Norway rats (BN) between the ages of 12 and 36 months (n=32). The results showed that aging-related signs of impairment did not appear before 18 months. Assessment scores for each of the measures subsequently increased with age. Variability in scores also increased with age. Comparing these results with the age-related changes in body weight and survival indicated the assessment method was more sensitive and provided unique data on the aging phenotype. Additional studies showed there was no difference in the aging trajectory of male Brown Norway rats from two different commercial suppliers. Scores for male BN-F344 hybrid rats (23-27 months) indicated less aging-related impairment than those obtained for BN rats in the same age range. On the other hand, scores for male Long Evans rats at 18 months of age indicated severe impairment, equivalent to that obtained in 30-month-old BN rats. The observational assessment provides...
an efficient means to monitor the health of aging rats and may be a useful tool in studies on age-related susceptibility to toxicants, drugs and disease. (This abstract does not necessarily reflect U.S. EPA policy.)

### 917 THE EFFECT OF ENRICHMENT ON THE INCIDENCE OF DIARRHEA IN CYMONOLGUS MACAQUES.


Diarrhea is the gastrointestinal disease most frequently encountered in captive non-human primates. The mechanisms underlying diarrhea in non-human primates are not well understood. Mauritian origin Cynomolgus macaques seem over-represented in the proportion of enteritis cases. Our site in South Texas instituted a Six Sigma project to determine the processes that might help us decrease the percentage of animals with diarrhea. We designed an enrichment program with measurable variables to determine if enrichment would have an effect on the percentage of animals getting sick with diarrhea. It was found that the morbidity in the Mauritius colony decreased significantly with the enrichment program.

### 918 USE OF MIXED-EFFECT MODELS TO EVALUATE MONKEY BODY WEIGHT VARIABILITY AND TRENDS IN BODY WEIGHT CHANGE DURING PRECLINICAL TOXICOLOGY STUDIES.


Cynomolgus monkeys are an important and widely used species in preclinical toxicology studies. In the per-life phase of study, body weight changes may be indicative of toxicity; however, trends in body weight change and body weight variability are often difficult to interpret due to small sample size and/or inter- and intra-animal variability. The present analysis utilizes mixed-effect modeling, which incorporates random and fixed effects into linear regression models, to evaluate intra-animal variability. The present analysis utilizes mixed-effect modeling, which incorporates random and fixed effects into linear regression models, to evaluate intra-animal variability. The present analysis utilizes mixed-effect modeling, which incorporates random and fixed effects into linear regression models, to evaluate intra-animal variability.

### 919 LUTEINIZING HORMONE (LH), FOLLICULAR-STIMULATING HORMONE (FSH), ESTRADIOL (E2), AND PROGESTERONE (P) LEVELS IN CYMONOLGUS MONKEYS AT MENSTRUAL ONSET AND MIDCYCLE.

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Determination of gonadotropins serum level (LH, FSH) and estradiol and progesterone are considered to be a reliable means of assessing ovarian function in the cynomolgus monkey. However, the best practice for yielding interpretive serum hormone levels when monitoring ovarian function in cynomolgus macaques needs thorough understanding of the changes in hormone patterns during the follicular and luteal phases of the menstrual cycle. The objective of this study was to determine whether serum levels at menstrual onset and mid-cycle will provide reliable hormone patterns to assess effects of drugs on ovarian function in cynomolgus monkeys. Twenty adult naïve female cynomolgus monkeys aged 4.1 -10.2 years, and weighing 3.2-5.9 kg were used in the study. The vaginal area of the monkeys were swabbed daily with a cotton-tipped applicator to detect menses. Serum levels of LH, FSH, E2, and P were evaluated via radioimmunoassay in serum samples collected at the onset of menstruation and at the calculated time of ovulation. The results obtained showed that LH (<0.8 ng/mL) and FSH (<1.6 ng/mL) values were below the limit of detection in 7 out of 20 and 17 out of 20 animals examined at the onset of menstrual period, respectively. The mean estradiol level at this stage was 29.5 ± 13.4 ng/mL and that of progesterone was 0.525 ± 0.14 ng/mL. At midcycle, most animals had LH and FSH values that were below limit of detection. Estradiol and progesterone levels appear to be slightly higher in the mid-cycle with mean of 33.45 ± 32.93 pg/mL and 2.65 ± 2.24 ng/mL, respectively. Most pre-clinical testing usually includes a single or two timepoints blood drawing for hormone determinations which seems not to be the best approach. Based on the results of this study daily drawing of blood for at least five days may be needed for gonadotropins because of a single-day surge during follicular phase and additional drawings to assess luteal phase to better design fertility study in female monkeys.

### 920 POSITIVE REINFORCEMENT TRAINING IN GOTTINGEN MINIPIGS.

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Positive reinforcement training (PRT) is a training method by which an animal is rewarded for showing desired behaviors and often a marker is used for accurately identifying the correct responses. The method has only been used to a very limited extent in laboratory animal species. We implemented PRT in a repeat dose toxicity study with intranasal dosing 10 times daily for one week in Göttingen minipigs. Three pigs were included in the study and each pig was trained for approx. 30 minutes per day for 14 days. Training was performed by a dedicated team of trainers, using the sound of a “clicker” as a conditioned reinforcer and GLP-certified dietary pellets as primary reinforcers (rewards). First, the clicker sound was associated with the primary reinforcer. This was followed by successive training steps using the clicker to mark correct responses until the complete desired behavioral response was reached. The fully trained pig voluntarily came forward, stepped onto a box (to elevate the pig), accepted the intranasal device and dosing in one nostril in a “freeze” position. All pigs learned the desired response prior to study start. The study was completed successfully with significant improved animal welfare and working conditions. We consider PRT a great potential in both the Göttingen minipig as well as in other laboratory animal species.

### 921 USE OF SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY (SD-OCT) FOR LONGITUDINAL EVALUATION OF SUBRETINAL INJECTIONS IN NON-HUMAN PRIMATES IN OCULAR TOXICOLOGY STUDIES.

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Subretinal administration of therapeutic agents can be advantageous to localize exposure close to a discrete region within the retina, or when a local depot delivery is required for slow release in the eye and is a common method of ocular delivery for gene therapy and cell-based products. SD-OCT allows for a non-invasive microscopic level cross sectional image of the retina in vivo, either as single sections or as volume scans. Six cynomolgus monkeys underwent subretinal injections. Under anesthesia, the eye was exposed with a lateral canthotomy. Under a surgical optical microscope, a 25G fiberoptic light source was inserted for visualization of the retina. A 41G needle, microextension tube and 1cc syringe were used to administer a 50 ul subretinal injection (bleb) of BSS into the superonasal region of the macula without entering the foveal region. OCT images were obtained at the time of injection and again every other week for 6 weeks, while the animals were under short anesthesia. There was a clear separation of the sensory retina from the RPE layer in the images obtained immediately post injection. The injection site itself was visible as a focal disruption of the sensory retinal layer. The height of the bleb was variable. By 2 weeks OCT I had demonstrated the retina had reattached in most places. Small retinal elevations were observed and occasionally a small fold was also noted. The injection site appeared as a disorganization of the retinal layers. At 4 and 6 weeks, the OCT images were able to show that no further degenerative changes developed in the retina in the area of the bleb, indicating the subretinal dosing procedure did not cause lasting physical changes that could interfere with the interpretation of toxicological changes of a test compound in the eye. Since multiple serial sections can be obtained in seconds, it was concluded that SD-OCT was a useful tool for the in-vivo monitoring of retinal changes when the retina is the site of administration.
A STUDY OF PHOTOTOXICITY FOLLOWING INTRAVENOUS ADMINISTRATION TO BALB/C MICE.


[Purpose] The in vitro 3T3 NRU phototoxicity test described in OECD guideline TG 432, but there is no in vivo phototoxicity test guideline. We reported (2008 SOT) on an oral administration in vivo phototoxicity test. Here, we evaluate the phototoxicity of ciprofloxacin hydrochloride (CPFX) and 8-methoxypsoralen (8-MOP) in Balb/c mice following intravenous administration because there is little information about in vivo phototoxicity studies. [Method] CPFX and 8-MOP were used as phototoxic substances. Male BALB/c mice, aged 6 weeks, were used. On the day before administration, the backs of the mice were shaved. The dosing preparations (50 and 100 mg/kg CPFX, and 5 and 10 mg/kg 8-MOP) were administered at 20 (CPFX) or 1 (8-MOP) mL/kg. Ten minutes after administration, the animals were irradiated with long-wave ultraviolet rays of approximately 20 J/cm2 with an Ultraviolet Irradiation Apparatus (Dermaray, M-DMR-50, Eisai Co., Ltd.). The thickness of both ears was measured with a dial thickness gauge, and the ears and back skin were observed before administration and at 0.5, 24, 48, and 72 hours after irradiation. Skin reactions (erythema and edema) at the ear and back were evaluated in accordance with the Draize method criteria. At 72 hours after irradiation, the ear and back skin were prepared for histopathology (Hematoxylin-Eosin stain). [Results] In the 50 and 100 mg/kg CPFX and 5 and 10 mg/kg 8-MOP groups, erythema or edema scored as 1 or 2 was observed at the irradiated sites. Ear thickness was increased in the 100 mg/kg CPFX group, and in the 5 and 10 mg/kg 8-MOP groups after irradiation. In histopathology, findings such as degeneration, edema, hemorrhage, and inflammatory cell infiltration were noted in the 100 mg/kg CPFX group and in the 5 and 10 mg/kg 8-MOP groups. In this study, positive reactions were clearly observed, and it was considered that the evaluation was possible in the intravenous administration in vivo phototoxicity test.

DEVELOPMENT OF A WEANLING PIG MODEL OF CUTANEOUS INJURY INDUCED BY CHLORINE VAPOR.

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Due to continued public concern for safety from chemical attacks, the Battelle Biomedical Research Center (BBBRC) and the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) have aligned research efforts to establish an anesthetized weanling pig model of superficial dermal and deep dermal cutaneous injuries induced by chlorine vapor. A chlorine vapor exposure system was fabricated and characterized to evaluate four lesion sites (each 3 cm in diameter) between the axillary and inguinal areas of the ventral abdomen in female Yorkshire- cross weanling pigs. Lesion assessment included clinical observations, digital photographs, size measurements, modified Draize scoring, reflectance colorimetry, and infrared thermographic images. Histopathologic evaluation of skin collected at 48 hours post-exposure included wound severity, wound depth, and percent of the total area involved. Saturated chlorine vapor at 2.9 g/L for 30 min did not induce dermal lesions on the ventral abdomen. Adding a wetted (simulated sweat) material (fabric or filter paper) onto the abdominal skin sites and exposing for 30 min to saturated chlorine vapor induced superficial dermal injuries and the material had dried. Using wetted filter paper discs placed on each exposure site for a saturated chlorine vapor exposure of 30 and 60 minutes, lesions were characterized and confirmed clinically and histologically at 48 hours, to be superficial dermal in severity. It is hypothesized that humidified chlorine vapor exposure experiments may be able to create a deep dermal lesion and these studies are currently underway.

EFFICACY AND TREATMENT OPTIMIZATION OF ANTI-INFLAMMATORY DRUGS APPLIED TO CUTANEOUS SULFUR MUSTARD INJURIES IN A WEANLING PIG MODEL.

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An agreement between the National Institute of Health (NIH), Battelle Biomedical Research Center (BBBRC), and the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) supports efficacy testing and treatment optimization of candidate drugs to improve wound healing against chemical agents of public concern. This study assessed the efficacy and treatment optimization of two NSAIDs (caposin 0.075% and diclofenac sodium 0.5%) with clobetasol propionate 0.05% applied topically to one site of two depths of injury, superficial dermal (SD) and deep dermal (DD), induced by sulfur mustard (HD). Fifty-four female, Yorkshire-cross pigs were randomly assigned to experimental groups representing all combinations of treatment duration (1, 3, or 5 days), daily number of applications (2, 3, or 4), and onset time (1, 2, or 4 h post-exposure) for each NSAID with steroid. Each of four ventral abdominal sites per pig had 400 μl of undiluted HD applied topically (2 sites 8 min exposure for SD injuries and 2 sites for 30 min DD injuries). Treatments were applied to one site per depth of injury at the respective time point post-exposure; the untreated sites served as within-animal controls. Site assessments included digital photographs, lesion size measurements, modified Draize Scoring, and non-invasive bioengineering techniques (reflectance colorimetry, infrared imagery, and transdermal water loss). Histopathologic evaluations were performed for wound healing parameters (re-epithelialization, dermal necrosis, and fibroplasia). Diclofenac sodium with clobetasol showed the most promise of healing HD-induced lesions. Both NSAID treatments provided improved healing of HD-induced lesions as the frequency and duration of treatment increased.

This work was supported by the U.S. Army Medical Research and Materiel Command under Contract W81XWH-05-D-0001, Task Order 0010.

TRI-SPECIES COMPARISON OF RESPIRATORY MECHANICS: BEAGLE DOGS, GOTTINGENS MINIPIGS AND CYNOMOLGUS MONKEYS.

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When the NOAEL is determined by respiratory safety pharmacology, follow-up studies are warranted and may include airway resistance and compliance. Respiratory mechanics in large animal species commonly used in safety pharmacology studies (Beagle dogs, Göttingens minipigs and Cynomolgus monkeys) were compared. Eighteen animals were used (3/sex/species) in an anesthetized model (propofol infusion) with pancuronium as a neuromuscular blocker and neostigmine for reversal. Parameters of respiratory mechanics were evaluated at baseline and at peak drug effect. The testing protocol included single-frequency forced oscillation analysis with a single compartment model in which resistance, elastance and compliance were calculated. Increasing doses of methacholine (2.32 to 263.5 μg/kg) were given by inhalation and the PD200, defined as the dose inducing a 200% increase in resistance, were calculated at baseline and after administration of albuterol by inhalation. Baseline PD200 was not significantly different in all 3 species. In minipigs, albuterol induced a mild to moderate increase (+33.4% ± 13.6%) in PD200 while a moderate to severe increase was noted in dogs (+284.8% ± 133.1%) and monkeys (+402.3% ± 90.8%). The increase in PD200 was significant in all species. Abundant scientific literature is available on respiratory mechanics in cynomolgus monkeys and Beagle dogs. In contrast, limited historical data is available in minipig respiratory mechanics. Our results suggest that dogs and monkeys were sensitive to albuterol administered by inhalation while minipigs were less sensitive.

CORRELATION OF FUNCTIONAL AND MORPHOLOGIC CHANGES IN A MOUSE MODEL OF EMPHYSEMA.


There is a growing interest in detecting functional decline in lung mechanics of animals with chronic obstructive pulmonary disease (COPD). Correlation of functional changes in pulmonary function, which include alterations in lung compliance and elastance, may serve as non-invasive early indicators of disease progression. In this study we induced emphysema, which is a core feature of human COPD along with chronic bronchitis, via estaline instillation. Lung function, morphometry, and histopathological evaluations were evaluated post dosing. Female C57BL/6 mice (N=8/10/group) were subjected to a single oro-tracheal instillation of estaline (40 μg/kg: 0.06 mL) or PBS (sham). At Week 1 and 3 postdosing, lung function was measured using the flexiVent® system. Lungs were removed, weighed, and the left lung was fixed for microscopic examination. Quantitative assessment of emphysema (airspace enlargement) was estimated by calculating the mean chord length using Image-Pro Plus®.

All mice were unremarkable postdosing and survived until the Week 3 sacrifice. However, the estaline-treated mice had higher dynamic compliance and lower tissue elastance compared to the sham control at both time points. This functional change correlated with histopathological observations of emphysema in treated mice which
was characterized by markedly enlarged alveoli which often had fragmented, occasionally hyaline or hyperecellular alveolar septa. The mean chord length was significantly higher in the treated mice at both Weeks 1 and 3. In summary, this study clearly correlated changes in lung function (lung compliance/elastance) against quantifiable structural damage (ephemysia) that remained similar over 3 weeks post elastase insult. (Funded by the Battelle Biological and Health Science Initiative)

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According to current regulatory guidelines, drugs intended for paediatric use require appropriate preclinical toxicity studies to be undertaken to ensure that there is no effect on development. To satisfy this requirement, a 13-week inhalation toxicity study on a novel bronchodilator was undertaken in Han Wistar rats from neonatal age (7 days old). The study design incorporated 6 dose groups (including 3 control groups) which required 89 dams, each with 5 male and 5 female pups, total 890 pups. Cross fostering was required to ensure an even sex distribution for each dam. Test aerosols were generated via airjet nebulisers. The animals were dosed by inhalation using a nose-only exposure technique for 30 minutes daily using specially designed inhalation tubes attached to a flow-past chamber. Aerosol particle size (MMAD) was controlled to 0.46-0.65 μm (the first 2-3 weeks) and 1.0-1.3 μm (the remainder of the study). There were 13 premature decedents in the study, many due to the behaviour of the dams (cannibalisation). Juvenile rats showed a higher respiratory minute volume than expected which was correlated with higher lung weights. Delayed sexual maturity in females was noted to be associated with a reduced body weight. The study revealed that juvenile rats can be dosed by the inhalation route from as early as 7 days old and that cross-fostering is feasible.

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According to current regulatory guidelines, drugs intended for paediatric use require appropriate preclinical toxicity studies to be undertaken to ensure that there is no effect on development. To satisfy this requirement, a 13-week inhalation toxicity study on a β2-agonist was undertaken in Beagle dogs from 15-19 days old. Thirty one male and 31 female puppies were allocated to 5 dose groups (including 3 control groups) which required 89 dams, each with 5 male and 5 female pups, total 890 pups. Cross fostering was required to ensure an even sex distribution for each dam. Test aerosols were generated via airjet nebulisers. The animals were dosed by inhalation using a nose-only exposure technique for 30 minutes daily using specially designed inhalation tubes attached to a flow-past chamber. Aerosol particle size (MMAD) was controlled to 0.46-0.65 μm (the first 2-3 weeks) and 1.0-1.3 μm (the remainder of the study). There were 13 premature decedents in the study, many due to the behaviour of the dams (cannibalisation). Juvenile rats showed a higher respiratory minute volume than expected which was correlated with higher lung weights. Delayed sexual maturity in females was noted to be associated with a reduced body weight. The study revealed that juvenile rats can be dosed by the inhalation route from as early as 7 days old and that cross-fostering is feasible.

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In response to a growing concern that bulk industrial chemicals could be used in terrorist attacks, the Battelle Biomedical Research Center (BBRC) and the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) have aligned research efforts to test medical treatments for chemically induced burns. To evaluate these treatments, an exposure system was developed, which controlled chlorine vapor concentrations to the skin of anesthetized weanling pigs. The exposure system was capable of simultaneously delivering chlorine vapor to four, 3-cm diameter exposure cups (two-cup pairs) placed over skin between the axillary and inguinal areas of the ventral abdomen. Each pair of exposure cups was independently controlled to permit two exposure durations per test. Hydrated conditions were also evaluated by including a wetted (simulated sweat) cloth or paper disks on skin sites (under the cup) or by the addition of water vapor directly to the chlorine atmosphere. Chlorine concentrations were generated by mixing 99.9% chlorine vapor with dried dilution air or nitrogen. Chlorine concentrations ranged...
from 0.1 to 2.9 g/L (saturated vapor concentration), and exposure durations ranged from 5 to 90 minutes. Real-time chlorine vapor concentrations were monitored between 200 and 800 nm by an in-line UV spectrometer. Saturated chlorine vapor challenges for up to 30 minutes did not induce significant dermal injuries, whereas saturated chlorine vapor with wetted material on the site for 30 to 60 minutes induced superficial dermal injuries. This study is ongoing, and results to date will be presented. This work was supported by the U.S. Army Medical Research and Materiel Command under Contract W81XWH-05-D-0001, Task Order 0010.

**932 ELECTRORETINOGRAPHY: COMPARISON OF A STANDARD PROTOCOL IN NEW ZEALAND WHITE RABBITS, BEAGLE DOGS, AND GÖTTINGEN MINIPINS.**
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Electroretinography (ERG) is often required to evaluate retinal toxicity of test articles after systemic or intravitreal administration. While basic ERG protocols in humans have been standardized, there is still significant variability between protocols used in different pre-clinical laboratories. The current study undertook evaluation of a standard ERG protocol in rabbits, dogs and minipins. ERGs were recorded using a computerized system with a Ganzfeld and contact lens electrodes referred to a sub-palpebra electrode and a ground. The protocol included a scotopic luminance-response curve analysis (-0.09 log cd/m² to 0.90 log cd/m²) with an average of 3 responses for each light intensity except for oscillatory potentials (OPs) where a single flash was also used. Photopic evaluations included a single flash (averaged response) followed by a flicker response at 50.3 Hz at 0.41 log cd/m². A minimum of 30 min of dark adaptation was used in all species. In dog, the same protocol was also evaluated after a light bleach (500W) to assess basal adaptometry curves. In scotopic evaluations, the amplitude of the a-wave was higher in the following species; minipins > dogs > rabbits at light intensities of ≥ 0.41 log cd/m² while the amplitude of the b-wave presented the same profile at light intensities of ≥ 0.41 log cd/m². Similarly, OPs were 194.6 ± 20.4, 108.3 ± 6.4, 62.9 ± 12.6 µV in minipins, dogs and rabbits, respectively. Light bleach induced a right shift of the luminance curve response and a decrease (~25%) of OPs. The ERG protocol evaluated could be useful as a standard testing in toxicology studies. Our study highlights intrinsic differences between the 3 species which may translate into different species sensitivity. Both single flash and averaged ERG tracings were adequate for evaluation of OPs, a-wave and b-wave without any evidence of a reduction in OPs with averaged flashes.

**933 SPARC-NULL MICE DEMONSTRATE REDUCED LUNG COLLAGEN AFTER ASBESTOS EXPOSURE.**

Pulmonary fibrosis involves the conversion of healthy lung tissue to scar tissue and affects roughly five million people worldwide. There is no known cure for the disease but only therapies to improve quality of life. Scar tissue formation must be interrupted in order to begin to provide any type of palliative therapy for fibrotic diseases. In this study, asbestos was used to induce pulmonary fibrosis in a mouse model and a candidate gene (Sparc) was identified. Sparc (secreted protein acidic and rich in cysteine) encodes a matricellular protein involved in tissue repair, extracellular matrix regulation, cellular proliferation, and cellular adhesion. The goal of this project was to determine the role of SPARC in fibrosis development after asbestos exposure, specifically how the lack of SPARC expression can influence collagen production. Our hypothesis was that SPARC was necessary for the fibrotic response to asbestos through an influence on collagen deposition in the lung. We had found that the expression of SPARC was increased in the lungs of C57BL/6j wild-type mice exposed to asbestos. This increase in expression correlated with higher collagen deposition in the lung. SPARC-null mice were used to demonstrate that although the inflammatory responses to asbestos were present, the absence of SPARC in these treated mice resulted in a reduction of the level of collagen deposition back to baseline. To determine the therapeutically potential of these findings, SPARC expression was reduced by small interfering RNA (siRNA) in wild-type mice already suffering from fibrosis. Measurements of collagen deposition in the fibrotic mice that received the SPARC siRNA vector showed a significant decrease in collagen accumulation when compared to those that did not receive the vector. Overall, these results indicate that expression of SPARC is a significant step in the development of lung fibrosis through the modulation of collagen deposition and therefore, SPARC may be a potential therapeutic target.

**934 HEALTH EFFECTS FOLLOWING EXPERIMENTAL EXPOSURE TO IRAQ PARTICULATE MATTER AND CIGARETTE SMOKE.**
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Several hundred thousand military and civilian personnel are deployed to the Middle East. Respiratory ailments including eosinophilic pneumonia possibly correlated to cigarette smoking are among the most frequent medical complaints. We sought to establish if inhaled sand-derived particulate matter (PM), in combination with cigarette smoke, contributed to the reported and observed conditions. The objectives of this study included examining the potential pathogenesis of airway inflammation and hyper-responsiveness associated with the complaints. In this study, male Sprague Dawley rats underwent a 6 week inhalation exposure to cigarette smoke (0.48 mg/m³, 3 h/d, 5 d/week), including two weeks of exposure to PM from Camp Victory in Iraq or silica (1 mg/m³, 19 h/d, 7 d/week) or air. Total inhaled smoke burden was approximately 500 mg/rat, and total PM approximately 3 mg/rat. Endpoints include behavioral, physiological and biochemical markers that signify pulmonary distress. The majority of observed effects result from smoking, and co-exposure to PM and smoke did not have synergistic effects. In the smoke-exposed groups, histopathology showed severe olfactory lesions and transformation to squamous cells in the nasal epithelium;bronchoalveolar lavage fluid showed elevation of neutrophils and changes in chitinase, kininogen and GST α. PM exposure induced minimal tracheal hyperplasia. α1-inhibitor III and pregnancy-zone protein were elevated following PM exposure, lowered following smoke exposure, and near baseline after co-exposure. Tissue metal levels were elevated by both smoke (Mn, Mo, Cd) and PM (Al, Si, V, Mn).

**935 MEASUREMENT OF LYMPHOCRYPTOVIRUS (LCV)-SPECIFIC IMMUNE RESPONSES IN CYMONULUS MONKEYS.**

There is a need to monitor immune function of non-human primates (NHP) for testing drugs with the potential for immune toxicity. Given that NHP are ubiquitously infected with the lymphocryptovirus (LCV) family of gammaherpesviruses, similar to human Epstein-Barr virus (EBV), we are developing a method to monitor T cell immune responses to LCV. LCV establishes a lifelong latent infection of B cells, which is controlled by T cells in immunocompetent individuals, but can result in posttransplant lymphoproliferative disease (PTLD) in the setting of immunosuppression. While the rhesus macaque sequence is known, the cyonomulus LCV counterpart is not currently available and limits studies in this commonly used primate species. Therefore, we generated a cyomonulus LCV-transformed B cell line (LC1) as an important source of antigen to study ex vivo LCV-specific immune responses. LC1s were expanded from long-term cultures of normal healthy cynomulus peripheral blood mononuclear cells (PBMCs) grown in the presence of cyclosorine. LCLs were positive for the B cell specific marker CD20. Immunohistochemistry and in situ hybridization analysis showed expression of EBV nuclear antigen 2 (EBNA-2) and EBV encoded nuclear RNA (EBER-1), respectively, hallmarks of LCV infection. Control PBMCs were negative for both EBNA-2 and EBER-1. LCL had > 10^8 copies of LCV D-polymerase per 10^6 cells by quantitative PCR. LCL-derived lysate elicited Interferon-gamma (IFNγ) production by LCV sero-positive PBMC, but not LCV sero-negative PBMC, as measured by IFNγ ELISPOT. Thus, an LCV-transformed LCL was obtained from cyonomulus monkeys and the lysate derived from the cell line can be used to stimulate and measure LCV-specific T cell immune responses ex vivo.

**936 8-OXOGUANINE DNA GLYCOSYLASE 1 (OOG1) IS REQUIRED IN THE DEVELOPING BRAIN.**
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The developing brain is vulnerable to be damaged by environmental toxins and endogenous metabolic products. Oxyguanine DNA glycosylase (OOG1) is the major DNA glycosylase that recognize DNA lesions and initiate the base excision repair.
pathway. Despite the importance of ogg1 in adult physiology, relatively little is understood about the role of ogg1 in the brain during embryogenesis. In this study, we describe for the first time the cloning and detailed expression pattern of ogg1 zebrafish genes. Sequence analysis revealed that zebrafish ogg1 proteins are 63% homologous to human orthologues. Based on RT-PCR analysis, ogg1 expression turns on at the very early stage, one cell (zygote). It stays in a relative high level during gastrulation, somite stage, and trough 24hpf to 8 days. Through RNA in situ hybridization we find that ogg1 is expressed in the whole brain zone, forebrain (diencephalon), midbrain (mesencephalon) and hindbrain (rhombomeres). Later the expression reaches the other developing nervous system, including spinal chord, notochord. Additionally, we found ogg1 also labeled the sinoatrial node. Using morpholino to knock down ogg1 expression, the brain size significant decreased. Bdu staining showed the profile cells in mo-injected embryos were almost gone. AO staining indicated the many brain cells underwent apoptosis. Compared to the wild type embryos, the mo embryos have less dividing cells in brain, evaluating by pH3 staining. Our expression analysis provided a starting point for studying the role of ogg1 in embryonic nervous system and suggested the potential role of ogg1 in protecting the developing brain. Further research on the function and regulation of ogg1 will help to understand the DNA repair mechanism in neural development and provide clinical therapy target.

### Evaluation of Genetic Diversity in Non-Human Primates Used in Research

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DNA analysis of targeted genetic loci has revealed genetic variation and similarities that underlie observed idiiosyncratic and generalized trends of responses to xenobiotics and lend efficiency to the pursuit of therapies for intractable human diseases. The non-human primate model has become important for the evaluation of safety and efficacy of many therapeutic biologics due to its genetic similarity with humans. Improved predictability and reproducibility of research data in non-human primate studies should be possible if the genetic variability of source non-human primates were better defined. We previously evaluated genetic variation between Chinese, Vietnamese, and Mauritian cynomolgus monkeys (Macaca fascicularis) at defined microsatellite loci. These evaluations provided evidence of less diversity in the Mauritian sourced animals and greater diversity in the other two sources. Possible hybridization among non-Mauritius Macaques was evaluated. We have expanded our evaluations to Indonesian and Philippine sourced animals as well as Chinese sourced Rhesus monkeys (Macaca mulatta) to expand investigations to other sources of animals and to begin assessing some neutral and non-neutral nuclear markers. Blood samples were collected from individuals of each geographic source. Microsatellite loci were amplified and sized using a capillary gel electrophoretic system. For other nuclear markers, genotypes were obtained using melt curve assays and sequencing of alleles. Observed population diversity indices (eg. Ho, A, R, FIS, FST) among groups were evaluated. The general trend of greater diversity in the non-Mauritius animals was again observed. These data provide valuable evidence that careful genetic profiling of small groups of animals is required to identify better animal selection and provide more consistent results with improved animal usage in non-human primate research studies.

### ICCVAM Recommendations for the Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Refine Ocular Toxicity Testing

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Eye injury is a leading cause of visual impairment in the U.S., with up to 50,000 new cases reported each year. To evaluate the potential of chemicals to cause eye injury is a leading cause of visual impairment in the U.S., with up to 50,000 new cases reported each year. To evaluate the potential of chemicals to cause eye injury, the protocol most widely accepted by regulatory agencies is based on the Draize rabbit eye test method. Since current ocular test guidelines state that users must ensure that the topical anesthetic does not affect test results, pain medications are often not used. However, for over 20 years CPSC has recommended pre-appli-
cation of a topical anesthetic for all rabbit eye toxicity studies. Therefore, ICCVAM recently conducted a comprehensive evaluation of the usefulness and limitations of routinely using topical anesthetics, systemic analgesics, and earlier more humane endpoints to minimize pain and distress in ocular toxicity testing. Following this evaluation, which included recommendations from an international independent peer review panel, ICCVAM concluded that a balanced preemptive pain management protocol should always be used when the Draize rabbit eye test is conducted for regulatory safety testing. This protocol should include pre-treatment with a topical anesthetic and systemic analgesic, and routine post-treatment with systemic analgesia. ICCVAM also recommends several additional humane endpoints that should be used to end studies earlier. To ensure timely and accurate detection of humane endpoints in ocular studies, ICCVAM recommends routine examination with a slit-lamp biomicroscope to characterize the nature, severity, and progression of any corneal lesions. ICCVAM also recommends routine observations for clinical signs of pain and distress at least twice daily, or more often if needed. Implementation of these ICCVAM recommendations should avoid or significantly reduce pain and distress associated with ocular safety assessments while continuing to support the protection of human health.

### Application of Magnetic Resonance Imaging (MRI) for Non-Invasive Local Assessment of Acute and Chronic Lung Response

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For the last couple of decades, researchers have been attempting to apply proton magnetic resonance imaging (MRI) to the characterization and diagnosis of pulmonary diseases. In this work we endeavored to distinguish diseased tissue from normal tissue in vivo in bleomycin-dosed rats by measuring MRI signal intensity (S0) and spin-spin relaxation time (T2) on a pixel-by-pixel basis. S0 measures water density, which is increased in both inflammation and fibrosis (vs healthy lung), and T2 is an indicator of water mobility, which is higher in inflammation. We also compared the MRI results to post-mortem measurements of lung collagen and water content. Male Sprague-Dawley rats were intratracheally dosed with a mixture of 0.2 mL saline (0.9% sodium chloride) and either 2.5 U/kg BW or 3.5 U/kg BW of bleomycin sulfate; control animals received saline only. Rats were imaged with MRI at 5 time points (pre-dose, then 1, 2, 4, and 7 weeks post-dose). Imaging was performed with a 2T horizontal-bore magnet and a Varian UnityPlus spectrometer. T2-weighted images were acquired with a slice selective spin-echo imaging sequence. Each image was separated temporally by a 6 ms delay. The planar resolution was 0.75 mm (lateral axis) x 1.0 mm (anteroposterior axis). Using measurements of both S0 and T2, bivariate normal confidence interval analysis was used to identify regions of non-normal tissue with ≥95% confidence. Results show that MRI is able to discern and locate abnormal tissue with statistical significance, when compared to normal lung images, and is well suited for following changes in the lung over time. Comparison of MRI results with post-mortem analysis of collagen and water content show comparable MRI sensitivity; however, MRI provides spatial information of disease that the post-mortem tests cannot. Important pre-clinical applications of this work include: animal screening to prevent blind sacrifice, monitoring response to inhaled toxicants, pharmaceutical testing, and facilitating targeted tissue harvesting.

### A Wireless Multisensor Telemetry Capsule for Monitoring Gastrointestinal Function in the Dog

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Alterations in gastrointestinal (GI) function are common side effects of many drugs, not limited to those administered orally. Changes in bowel habits and gastric irritation are the most commonly reported side effects in humans. Preclinically, GI disturbances such as intestinal and/or colonic motility, gastric emptying/sorption are generally assessed in a number of usually rodent model systems, to provide a safety pharmacology evaluation. In these model systems, measurements are usually performed in the context of a non-calooric meal, use quite large cohorts of animals and require terminal sacrifice. SmartPill® is an ingestible, wireless motility capsule (WMC) which received approval by the U.S. FDA for GI function assessment in humans. SmartPill® provides ambulatory testing for GI pressure, temperature, luminal pH, gastric emptying time, combined small and large intestine transit time, and total GI transit time. This device, although extensively tested in humans, has not been fully evaluated in dogs. The objective of this study was to assess the feasibility of measuring GI transit time and gastric pH in dogs in safety pharmacology studies. Four female Beagle dogs were used. Following overnight fasting, animals were fed with 100 g of canned food. The capsule was administered immediately following food consumption. Dogs were equipped with pocketed jacket to hold the data receiver, and animals were returned to their pens. Dogs were monitored until the capsule was expelled in the feces. In order to evaluate the ability of the SmartPill® to reliably detect GI effects of drugs, animals were treated with a pro-kinetic (erythromycin), an anti-kinetic (morphine) and an anti-acid (pantoprazole). Our results show that the SmartPill® technology is a novel, noninvasive method for monitoring GI function that may complement rodent data in order to better predict GI function of drugs in humans.
The science of toxicology and its associated policy and societal issues, is of paramount global concern. With the use of toxic chemicals increasing in all corners of the world, particularly in developing countries, toxicology is stepping into the forefront as a major science that can help in our understanding of environmental problems and provide solutions for protecting public health. The multi-lateral, multi-lingual, web-based, World Library of Toxicology (WLT as www.wltox.org) was developed to facilitate this exchange by decreasing the barriers to sharing information among countries and building capacity within countries. Volunteer Country Correspondents share their country-specific knowledge of toxicology and public health. The Correspondents build their respective country pages by contributing links to resources, such as relevant governmental and non-governmental organizations, university or other training programs, poison control centers, professional associations, databases, books, journals, general country information and other topics. The WLT uses a modified Wiki based technology that provides the Country Correspondents with control of their individual country content, allowing for rapid and continuous expansion of information. There are currently some 50 countries represented and we actively recruit new country correspondents as part of our efforts to expand the program. The WLT is a joint project the International Union of Toxicology, the National Library of Medicine, the Institute of Neurotoxicology & Neurological Disorders, and supported by Toxipedia (www.toxipedia.org). The WLT was launched in South Africa at the 7th Congress for Toxicology in Developing Countries in September 2009 and has had over 2,500 unique visitors from around the world during the month following its release. The WLT offers a unique way to share information, acknowledge our ethical responsibilities, and build global capacity in toxicology research, application, and training for a healthy and sustainable world.

**941 CREATING A VIRTUAL GLOBAL TOXICOLOGY VILLAGE – THE WORLD LIBRARY OF TOXICOLOGY (WLT).**

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Paraphrasing, Spiderman’s Uncle Ben, “With great knowledge comes great responsibility”. Toxicologists are confronted with a complex mixture of ethical, legal, social, and professional issues during their career, which have become more pronounced with the expanded knowledge about the health effects of chemicals and evolution of society. Data on the effects of chemical and physical agents on human health and the environment can have significant finical implications as well individual and societal implication. It is thus important for toxicologist to give careful consideration to values and ethics that underlie scientific research and decision making. The fundamental principles that an ethical toxicologist should consider can be summarized as: 1) dignity, which includes the respect for the autonym of human and animal subjects; 2) veracity, an adherence to transparency and presentation of all the facts so all parties can discover the truth; 3) justice, which includes an equitable distributions of the costs, hazards, and gains; 4) integrity, an honest and forthright approach; 5) responsibility, an acknowledgement of responsibility and accountability to all parties involved; and 6) sustainability, consideration that actions are sustainable over a long period of time. SOT has a robust code of ethics that includes many of these elements and requests that society members adhere to the code. Ideally, the ethical toxicologist must move beyond adherence to the legal regulator requirements or best practices and be guided by a deeper ethical foundation grounded in a consistent philosophy of basic values and principles. This presentation is based upon a recently published chapter in General and Applied Toxicology – 3rd Edition, entitled Ethical, Legal, Social, and Professional Issues in Toxicology by Steven G. Gilbert and David L. Eaton.

**942 THE ETHICAL TOXICOLOGIST – CONSIDERATIONS OF ETHICAL, LEGAL, SOCIAL, AND PROFESSIONAL ISSUES IN TOXICOLOGY.**

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The interest of engaging undergraduates in the field of toxicology may benefit from utilizing a novel approach: service-learning (SL). SL is gaining momentum in institutions of higher education due to the positive influence on students for leadership, values, and future career choices. The purpose of this project was two-fold: to engage undergraduates in mentored toxicology research while serving the community; and to foster a community-university partnership by performing exposure assessment, utilizing blood lead screening. A research pod was established, comprised of three undergraduate students and one faculty member who provided training and mentoring. This was an IRB approved project for lead screening in community centers serving special needs children. Capillary blood lead levels were measured in mothers and children (n=45), and matched with self-reported ethnicity, and zip codes of residence and community centers. Education and information on lead and poison prevention were provided by family interviews and public health education materials. Students were educated in scientific research methods, including: literature reviews, data collection, storage, measurement, and analysis. Differences in laboratory methods of capillary and venous blood lead collection and results were also studied. Follow-up surveys were provided to parents, students, and participating community centers. Parents were followed up to insure healthcare provider notification of results and to assess further questions on toxic exposures and community resources. Student self-evaluation was performed at the completion of the research pod and indicated an overwhelmingly positive experience, with suggestions for areas of future toxicology research projects and student involvement in applied toxicology. Community center feedback was also positive, with interest in future partnerships with university students and research of toxic and environmental exposures. SL should be considered as a tool for engaging undergraduates in toxicology research and education.

**943 USING RESEARCH TO DEVELOP A WEB-BASED PORTAL FOR TOXICOLOGICAL INFORMATION.**


The Centers for Disease Control and Prevention’s (CDC) National Center for Environmental Health (NCEH) and the Agency for Toxic Substances and Disease Registry (ATSDR) are charged with protecting and maintaining the environmental health of Americans and reducing and preventing their exposure to toxic chemicals. The agencies use the Internet as a primary communication channel for conveying information. Therefore, the ability to find information easily and quickly is critical. Usability testing and evaluation offer the best approach to gauging the impact of the Web as a communication channel. In 2006 NCEH/ATSDR contracted for a series of Web usability studies that used close interviews to assess the two agency Web sites. Qualitative assessments gathered feedback on the ease of finding and understanding information and the quality of information. Quantitative assessments captured success rates, average time on task, and users’ paths. Responding to feedback from industry and academic scientists, public health professionals, and community members, NCEH/ATSDR developed a Toxic Substances Portal. This innovative content delivery mechanism organizes and communicates toxicological information from various sources within the agency. The first version was released in 2007, and a new version with a new template was published in 2008. In 2009, NCEH/ATSDR focused a Web usability study on the portal. NCEH/ATSDR now is implementing the changes outlined in the research findings. Research findings and lessons learned from this project enabled NCEH/ATSDR to improve organization of their Web sites’ toxicological information to match audience preferences. Visitors to the NCEH and ATSDR Web sites now can find the information they need quicker and easier. The findings from these studies can be applied to other projects and help professionals understand how Web visitors search for information about environmental health and toxicology. The lessons learned can also guide Web communication strategies for other organizations.

**944 TOXICOLOGY AND UNDERGRADUATE EDUCATION: AN APPROACH THROUGH RESEARCH AND SERVICE-LEARNING.**


Service-learning (SL) is gaining momentum in institutions of higher education due to the positive influence on students for leadership, values, and future career choices. The purpose of this project was two-fold: to engage undergraduates in mentored toxicology research while serving the community; and to foster a community-university partnership by performing exposure assessment, utilizing blood lead screening. A research pod was established, comprised of three undergraduate students and one faculty member who provided training and mentoring. This was an IRB approved project for lead screening in community centers serving special needs children. Capillary blood lead levels were measured in mothers and children (n=45), and matched with self-reported ethnicity, and zip codes of residence and community centers. Education and information on lead and poison prevention were provided by family interviews and public health education materials. Students were educated in scientific research methods, including: literature reviews, data collection, storage, measurement, and analysis. Differences in laboratory methods of capillary and venous blood lead collection and results were also studied. Follow-up surveys were provided to parents, students, and participating community centers. Parents were followed up to insure healthcare provider notification of results and to assess further questions on toxic exposures and community resources. Student self-evaluation was performed at the completion of the research pod and indicated an overwhelmingly positive experience, with suggestions for areas of future toxicology research projects and student involvement in applied toxicology. Community center feedback was also positive, with interest in future partnerships with university students and research of toxic and environmental exposures. SL should be considered as a tool for engaging undergraduates in toxicology research and education.
Expression of cobalamin transporter TCN2 was reduced by 50%, although expression of folate transporters such as CYP19A1 and SLC46A1 were either unchanged or reduced and folate receptor (FOLR1) transcripts were moderately increased (157%). However, expression of transporters known to deplete intracellular folate, such as ATP Binding Cassette C1 (ABCC1), ABCC3 and ABCC5 were all markedly increased. Interestingly, flow cytometry revealed a broader shift in efflux capacity of As treated cells toward Organic Anion Transporters (OAT) and ABC type of efflux pumps. This suggests that a modification of efflux capacity might be responsible for depletion of As-induced folate and, potentially, cobalamin, and subsequent disruption of the methyl metabolism. Expression of the proto-oncogene MYC, which is known to up regulate expression of OAT and ABC in cancer, was also increased (2-3 fold). These data suggest that the DNA methylation changes found in As treated RPE-1 cells are an indirect consequence of the survival adaptation to As involving increased efflux, that results in these long term cumulative detrimental effects.

We have recently reported that phosphorylating arsensolate enzymes promote thiol-dependent As reduction because they convert AsV into a arsenylated product in which the arsenic is more easily reduced by thiols to arsenite (AsIII) than in inorganic AsV. We have also shown that isolated mitochondria can rapidly reduce AsV in a process that depends on intact oxidative phosphorylation and presence of GSH in the mitochondria. Thus, these organelles might reduce AsV because mitochondrial ATP synthase, using AsV instead of phosphate, oxidatively arsenylates ADP to ADP-AsV, which in turn is readily reduced by GSH. To test this hypothesis we first examined whether the RNA-degrading enzyme PNPase, which can split polyadenylate (poly(A)) by arsensylation into units of AMP-AsV (a homologue of ADP-AsV), could also promote reduction of AsV to AsIII in presence of thiols. Indeed, bacterial PNPase markedly facilitated formation of AsIII when incubated with polyA, AsV and GSH. PNPase-mediated AsV reduction was dependent on arsenosylation of polyA and presence of a thiol compound. While various thiols did not influence the arsenolitic yield of AMP-AsV, they differentially promoted the PNPase-mediated reduction of AsV, with GSH being the most effective. Circumstantial evidence was obtained to show that AMP-AsV formed by PNPase is more reducible to AsIII than GSH, while the AsV-adenylate (polyA) by arsenylation into units of AMP-AsV (a homologue of ADP-AsV). Cell viability was evaluated after exposure to sodium arsenite using WST-8. Concentration of total cellular arsenic was measured by inductively coupled plasma mass spectrometry (ICP-MS) after acid digestion. Speciation analyses of arsenic in cellular fractions and cultured medium were performed using HPLC cells that are known to express hAS3MT and T-REx CHO cells with or without induction of hAS3MT. After harvesting the cells, arsenic metabolites were extracted and the exerts were analyzed by HPLC-ICP-MS using a reversed phase column. Cell viability of the tet-induced cells was significantly lower than that of untreated cells after 24 h-exposure to arsenic. Concentration of total cellular arsenic in the tet-induced cells was significantly greater than that of untreated cells. Most of cellular arsenic in arsenite-exposed HePG2 cells appeared to be protein-bound monomethylarsonic acid. A trace amount of protein-unbound dimethylarsonic acid was also detected. In contrast, most of cellular arsenic appeared to be protein-unbound dimethylarsinic acid and protein-bound monomethylarsonic acids were detected only as minor metabolites in the tet-induced cells. These results suggest that hAS3MT enhances the cytotoxic effects of arsenic by generating highly reactive protein-bound arsenic species. We also found that T-REx CHO cells accumulated a larger amount of arsenic when hAS3MT was induced by tetracycline, suggesting an implication of methylation in the cellular retention and toxicity of arsenic.

Environmental exposure to inorganic arsenic has been widely associated with a broad array of diseases. Individual variability in human arsenic metabolism has been shown to influence arsenic-induced disease risk. Among the factors that have been shown to be associated with variability in arsenic metabolism, genetic variants in arsenic (3+) oxidation state) methyltransferase (AS3MT), the key gene in the metabolism of arsenic, have been associated with increased arsenic methylation efficiency. In addition, it has been reported that several of the AS3MT single nucleotide polymorphisms (SNPs) are in linkage disequilibrium (LD). The existence of a cluster of high LD represents a challenge; as such genetic associations cannot be unambiguously assigned to AS3MT. To characterize the extent of LD within this chromosomal region, we genotyped 46 SNPs in a genomic region of chromosome 10 that included AS3MT in 327 arsenic exposed subjects from Mexico. Pair-wise LD analysis showed strong LD, with a mean r2 of 0.82 for these 46 polymorphisms. This region of high LD was surprisingly large, spanning a 347,000 base region that includes AS3MT and 4 other genes. Genetic association analysis in these subjects showed that all linked polymorphisms tested in this region except one were significantly (p < 0.05) associated with arsenic methylation efficiency, measured as the dimethylarsinic acid : monomethylarsenic acid ratio. This unusually large LD region adds complexity to genetic association studies of AS3MT. Not only can the genetic association with arsenic methylation efficiency not be unequivocally assigned to a single SNP within AS3MT, there is no certainty that AS3MT is the gene responsible for the association. This information should be carefully considered in future association studies seeking to identify the causal genetic polymorphism or polymorphisms that drive the human variation in arsenic metabolism. (Funded by ES06694 and ES04940).
ARSENITE DOWN-REGULATES THE CARCINOGEN ACTIVATING ENZYME CYTOCHROME P450 1A1 AT THE TRANSCRIPTIONAL AND POST-TRANSLATIONAL LEVELS.

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Arsenic acid hydrocarbon receptor (AhR) ligands, typified by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and heavy metals, typified by arsenite (As3+), are common environmental co-contaminants with important toxicological consequences. As such, their molecular interaction may disrupt the coordinated regulation of the carcinogen activating enzyme Cytochrome P450 1A1 (CYP1A1). Therefore, we examined the effect of co-exposure to As3+ and TCDD on the expression of the AhR-regulated gene, CYP1A1, at each step of the AhR signal transduction pathway. For this purpose human HepG2 cells were incubated with different concentrations of As3+ in the presence of 1 nM TCDD. CYP1A1, and heme oxygenase (HO-1) mRNA expression was measured using real-time PCR. Protein expression and activity levels were measured using Western blot analysis, and 7-ethoxyresorufin O-deethylase activity, respectively. As3+ effect on CYP1A1 mRNA decay was assessed using actinomycin-D-chase experiment. Our results showed a dose-dependent decrease in the TCDD-mediated induction of CYP1A1 mRNA, protein, and catalytic activity levels upon treatment with As3+. Looking at the transcriptional level, our results revealed that As3+ significantly inhibited the TCDD-mediated induction of AhR-dependent luciferase reporter gene expression. Moreover, As3+ failed to attenuate CYP1A1 mRNA stability, yet with a tendency towards increasing the transcripts half-life. In addition, As3+ increased HO-1 mRNA in a dose-dependent manner, and this increase coincided with further decrease in the CYP1A1 catalytic activity. Upon using a competitive HO-1 inhibitor, tin mesoporphyrin (SnMP) or transfecting the HepG2 cells with HO-1 siRNA there was a partial restoration to the TCDD-mediated induction of CYP1A1 catalytic activity. In conclusion, this study provides the first evidence that As3+ acts to down-regulate CYP1A1 gene expression through transcriptional and post-translational mechanisms, probably through inducing HO-1. Supported by NSERC Discovery Grant RGPIN 250139-07.

N6 ADENINE-SPECIFIC DNA METHYLTRANSFERASE 1 MAY PLAY A ROLE IN ARSENIC METABOLISM AND TOXICITY.

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Consumption of drinking water contaminated with high levels of inorganic arsenic (iAs), an established human carcinogen, is a worldwide public health concern. In humans, iAs is converted to methylated arsenic metabolites mainly by arsenic (+3 oxidation state) methyltransferase (AS3MT) in a multistage process. Recently, we showed that the MTO2 gene is essential for optimal growth of yeast in the presence of iAs. The human N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) is a putative methyltransferase orthologous to the yeast MTO2. As a putative gene, N6AMT1 functions are largely unknown. We showed that N6AMT1 is expressed in many human tissues with varied expression levels. To further explore the roles of N6AMT1 in arsenic toxicity, we highly enhanced its expression in an uroepithelial cell, UROtsa, which is defective in methylating arsenic due to the loss of AS3MT. Results indicate that while DNA methylation remains relatively stable in 12 wk exposure. The persistence of DNA damage in MMAIII-exposed cells was demonstrated in the presence and after subsequent removal of MMAIII. In the presence of MMAIII, UROtsa cells demonstrated a sustained-increase in DNA damage following 12 wk exposure. The persistence of DNA damage in MMAIII-exposed cells was on biological specimens from populations exposed to arsenic. We recently reported decreased β-defensin-1 (HBD1) peptides in urine from men in Nevada and Chile exposed to arsenic in drinking water (Hegeduš et al., 2008). Subsequent in vitro analysis revealed suppressed HO-1 mRNA following arsenic treatment, providing evidence that HBD1 may be a biomarker of response to arsenic. To further explore these findings, we investigated effects of arsenic on HBD1 expression in immortalized human cells derived from skin, kidney and bladder, which are target tissues of arsenic toxicity. First, we performed RT-PCR analysis to measure HBD1 expression, and found highest and lowest expression in kidney and bladder cells, respectively. We then determined effects of arsenic on HBD1 mRNA and found no effect on bladder cells since basal expression was too low. The keratinocytes and kidney cells were therefore used in subsequent experiments. Our data reveal a dose- and time-dependent decrease in HBD1 mRNA following treatment with As3+ or its more toxic metabolite, MMAIII, and the kidney cells were more sensitive to both species. Taken together, these data suggest that decreased urinary HBD1 measured in our epidemiological studies is most likely a direct result of decreased production in the kidneys, rather than the bladder, resulting from arsenic exposure. We are currently investigating the mechanism of HBD1 suppression. HBD1 is an antimicrobial peptide constitutively expressed in skin and mucosal epithelia of multiple tissues, and functions in both innate and adaptive immune responses. HBD1 down-regulation has been reported in various cancers, suggesting that HBD1 may be a tumor suppressor gene. It is therefore plausible that suppression of HBD1 may play a role in arsenic-related carcinogenesis.

INVESTIGATING BETA-DEFENSIN-1 DOWN-REGULATION IN ARSENIC TOXICITY.

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Arsenic is a human carcinogen and a major drinking water contaminant for millions of people worldwide. To gain mechanistic insight into arsenic toxicity and identify novel biomarkers of response, our laboratory conducted proteomic analysis on biological specimens from populations exposed to arsenic. We recently reported decreased β-defensin-1 (HBD1) peptides in urine from men in Nevada and Chile exposed to arsenic in drinking water (Hegeduš et al., 2008). Subsequent in vitro analysis revealed suppressed HO-1 mRNA following arsenic treatment, providing evidence that HBD1 may be a biomarker of response to arsenic. To further explore these findings, we investigated effects of arsenic on HBD1 expression in immortalized human cells derived from skin, kidney and bladder, which are target tissues of arsenic toxicity. First, we performed RT-PCR analysis to measure HBD1 expression, and found highest and lowest expression in kidney and bladder cells, respectively. We then determined effects of arsenic on HBD1 mRNA and found no effect on bladder cells since basal expression was too low. The keratinocytes and kidney cells were therefore used in subsequent experiments. Our data reveal a dose- and time-dependent decrease in HBD1 mRNA following treatment with As3+ or its more toxic metabolite, MMA III, and the kidney cells were more sensitive to both species. Taken together, these data suggest that decreased urinary HBD1 measured in our epidemiological studies is most likely a direct result of decreased production in the kidneys, rather than the bladder, resulting from arsenic exposure. We are currently investigating the mechanism of HBD1 suppression. HBD1 is an antimicrobial peptide constitutively expressed in skin and mucosal epithelia of multiple tissues, and functions in both innate and adaptive immune responses. HBD1 down-regulation has been reported in various cancers, suggesting that HBD1 may be a tumor suppressor gene. It is therefore plausible that suppression of HBD1 may play a role in arsenic-related carcinogenesis.

EXPOSURE OF A HUMAN BLADDER CELL LINE TO SHORT-TERM, LOW-LEVEL MONOMETHYLARSONIC ACID PRODUCES CRITICAL AND IRREVERSIBLE EVENTS RESULTING IN MALIGNANT TRANSFORMATION.

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Arsenic is a known human bladder carcinogen; however, the exact molecular mechanisms underlying arsenical-induced bladder carcinogenesis are unknown. Exposure of the non-tumorogenic human urothelial cell line, UROtsa, to 50 nM of the arsenic metabolite, monomethylarsonic acid (MMAIII), for 52 wk resulted in malignant transformation. To focus research on the critical period of MMAIII-induced malignant transformation in UROtsa cells, the goal of this research was to evaluate the minimum duration of 50 nM MMAIII exposure necessary to induce malignant transformation of UROtsa cells. Results demonstrate that hyperproliferation of UROtsa cells first occurs after 12 wk of MMAIII exposure. Initial signs of anchorage-independent growth occurred after 12 wk, with a continued increase in colony formation when these cells were cultured for an additional 12 or 24 wk without MMAIII exposure. Furthermore, 12 wk MMAIII-exposed UROtsa cells cultured for an additional 24 wk without MMAIII demonstrated significant tumor formation when injected into SCID mice. To assess potential underlying mechanisms associated with the early changes that occur during MMAIII-induced malignant transformation, DNA methylation was assessed in target gene promoter regions. Results indicate that while DNA methylation remains relatively stable in 12 wk MMAIII-exposed UROtsa cells; aberrations are detected in these cells after an additional 12 and 24 wk in culture without MMAIII exposure, coincident with the emergence of a tumorigenic phenotype. These data provide evidence that 50 nM MMAIII is capable of “ imprinting” UROtsa cells following 12 wk of MMAIII exposure and that malignant transformation is irreversible upon removal of MMAIII. The current data provide a foundation for future mechanistic studies focused on the biological changes that occur during these critical time points and exemplifies the carcinogenic potential of MMAIII.

SHORT-TERM, LOW-LEVEL EXPOSURE OF A HUMAN BLADDER CELL LINE TO MONOMETHYLARSONIC ACID DAMAGES DNA AND ALTERS REPAIR ACTIVITY.

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Exposure to 50 nM monomethylarsonic acid (MMAIII) for 12 wk is sufficient to cause malignant transformation of the non-tumorogenic human urothelial cell line (UROtsa). MMAIII has been shown to cause a genotoxic insult; however, the exact molecular mechanisms involved are unknown. UROtsa cells exposed to 50 nM MMAIII were used to study DNA damage and the alteration of DNA repair in the presence and after subsequent removal of MMAIII. In the presence of MMAIII, UROtsa cells demonstrated a sustained-increase in DNA damage following 12 wk exposure. The persistence of DNA damage in MMAIII-exposed cells was...
assessed after a 2 wk removal of MMAIII. Previously exposed UROtsa cells demonstrated a decrease in DNA damage compared to UROtsa cells in the presence of MMAIII; however, prolonged previous exposure resulted in DNA damage levels greater than control UROtsa. ROS were demonstrated to be involved in the generation of DNA damage determined through the incubation of ROS scavengers with MMAIII-exposed UROtsa cells. PARP is a key repair enzyme in DNA single strand break repair. In MMAIII-exposed UROtsa there was a decrease in PARP activity, suggesting the presence of MMAIII is inhibiting the increase in PARP activity. Interestingly, PARP gene expression increases in a time-dependent manner in MMAIII-exposed UROtsa cells. When MMAIII was removed from UROtsa cells, PARP activity increased significantly, coinciding with a decrease in DNA damage. MMAIII-exposed UROtsa cells also have increased levels of DNA double strand breaks, demonstrating the severity of MMAIII-induced genomic insult. These data demonstrate that short-term, low-level exposure of UROtsa cells to 50 nM MMAIII results in: the induction of DNA damage that remains elevated upon removal of MMAIII; increased levels of ROS that play a role in MMAIII-induced DNA damage, decreased PARP activity in the presence of MMAIII, and the formation of double strand breaks.

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955 CYTOTOXICITY OF ARSENICALS IN PRIMARY HUMAN EPITHELIAL CELL CULTURES.
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Epidemiological data has established a connection between the development of bladder cancer and chronic arsenic exposure. Acute effects of arsenic exposure have been well characterized with immortalized human urothelial cells derived from the lining of the ureter. In addition, chronic exposure of these immortalized cells to arsenicals has led to their malignant transformation. In this study, primary human bladder epithelial (HBE) cells were purchased from CELLInTEC (www.celln-tec.com) and were maintained in a proprietary culture media designed to prolong growth, proliferation, and delay differentiation. Preliminary studies showed a similar sensitivity to acute exposure of sodium arsenite and monomethylarsenous acid [MMA(III)], a highly toxic metabolite of arsenite, when compared to immortalized urothelial cells. When HBE cells were differentiated by addition of 1 mM calcium [MMA(III)] results in: the induction of DNA damage that remains elevated upon removal of MMAIII; increased levels of ROS that play a role in MMAIII-induced DNA damage, decreased PARP activity in the presence of MMAIII, and the formation of double strand breaks.

956 LOW-DOSE ARSENIC DECREASES THE MIGRATION OF DENDRITIC CELLS.
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Arsenic (As) exposure is a significant worldwide environmental health concern and chronic As exposure via contaminated drinking water has been associated with an increased incidence of pulmonary and other serious diseases. We recently reported that exposure of mice to environmentally relevant levels (100 ppb) of As in the drinking water significantly altered the innate immune response within the lung, leading to a severely compromised response to Influenza A (H1N1) infection, in part due to a decrease in the migration of dendritic cells from the lung to the lymph node early in the course of infection. Given that this migration is a critical component to the initiation of a proper immune response, our findings led us to investigate the effect of As exposure on the migration capabilities of mouse bone marrow-derived dendritic cells in culture. We found that exposure of cells to As exposure significantly impaired their migration towards a chemoattractant in a transwell assay. The effect was evident at concentrations well below 10 ppb, the current U.S. EPA drinking water standard. Our results indicate that As effects on dendritic cell migration may be a significant contributor to the overall infection response we observed in our mouse influenza model. Moreover, these results suggest that chronic arsenic exposure, even at concentrations 100-fold below the current EPA limit may affect critical aspects of the innate immune response, contributing to altered disease risk. (NIH-NIEHS SRP P42 ES07373 F2)

957 LOW DOSE ARSENIC HAS PRO-ATHEROGENIC EFFECTS ON MACROPHAGES IN VITRO AND IN A MURINE MODEL.
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Arsenic exposure is linked epidemiologically to increased cardiovascular disease, such as atherosclerosis. However, the mechanism by which arsenic enhances atherosclerosis and the minimal dose at which arsenic exposes subjects to risk of this disease is unknown. Therefore, we assessed the effects of a low-to-moderate exposure to arsenic on monocyte adhesion and migration, and on lesion formation in the vascular walls in a murine model. Monocytes/macrophages are key players in the early stages of atherosclerotic plaque lesion formation. They bind to the activated endothelium of vessels and migrate under the endothelial cells where they become lipid laden and form foam cells. Therefore, we first tested whether arsenic modulates the adhesion of monocytes to the adhesion molecule VCAM-1, normally found on activated endothelial cells. Our results showed that: 1) adhesion to VCAM-1 is altered by arsenic exposure; 2) arsenic modulates monocyte expression of β1 integrin, the molecule that binds VCAM-1; and 3) the low-to-moderate doses of arsenic (10-200 ppb) increase adhesion more than the higher dose (1 ppm). In addition, using an organ culture system, we found that arsenic exposure increased the adhesion of murine monocytes to the endothelium of mouse carotid arteries. Next, we showed that as low as 10 ppb of arsenic enhanced macrophage migration in a scratch assay. Finally, we exposed ApoE−/− mice (a murine model of atherosclerosis) to two doses of arsenic for 8 or 13 weeks. Interestingly, we observed more extensive atherosclerotic lesions in mice exposed to the low dose (200 ppb) of arsenic, compared to either the control or moderate dose (1 ppm), at both time points. These results suggest that arsenic enhances atherosclerosis by increasing macrophage adhesion and migration. Significantly, our data shows that arsenic enhances atherosclerotic lesion development at lower doses than previously reported and may thus be a greater health risk than previously anticipated.

958 ROLE OF IL-8 IN MONOMETHYLARSENIC ACID-INDUCED HUMAN BLADDER CELL MALIGNANT TRANSFORMATION.
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Interleukin 8 (IL-8) is over expressed in various human cancers and its levels in blood correlates with tumor stage, disease progression, and recurrence. After binding to its receptors (CXCR1 and CXCR2), IL-8 activates a variety of intracellular pathways, promoting cell proliferation, tumor growth, and metastasis. In transitional cell carcinoma of the bladder, IL-8 is a mediator of angiogenesis, tumorigenicity, and metastasis. The toxic metabolite of arsenite, monomethyl arsenuic acid [MMA(III)] has been shown to malignantly transform UROtsa cells. In this study the role of IL-8 in arsenic-related bladder cancer development was determined using an immortalized urothelial cell line (UROtsa). Transformation and CXCR1 expression as well as IL-8 related intracellular pathways were profiled in different phases of MMA(III)-induced cell transformation. IL-8 was over-produced in UROtsa cells after 3 mo of exposure to 50 mM MMA(III) coinciding with a faster growth rate. These cells also showed an increased expression of CXCR1 and a sustained AKT, p38 and ERK1/2 over-activation. When the chronic MMA(III)-exposed cells were stimulated with 100ng/ml IL-8, the cells showed a faster growth rate, possibly as a result of their increased CXCR1 expression. These results suggest that IL-8 and CXCR1 over-expression play a relevant role in MMA(III)-induced UROtsa cell transformation. (NIH ES07490, ES06694, US-Mexico Binnational Center, CONACyT 91679).

959 CHRONIC EXPOSURE TO ENVIRONMENTAL LEVELS OF ARSENIC INCREASE NFκB ACTIVATION AND ALTER ENDOTHELIAL FUNCTION.
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Exposure to environmental arsenic has been associated with several diseases, including cancer, cardiovascular disease and diabetes, making arsenic exposure a worldwide health concern. Microarray data from our laboratory has suggested that environmental levels of arsenic result in a general downregulation of gene expression in isolated endothelial cells. The overall goal of this project was to determine whether...
inflammatory signaling is altered by chronic exposure to environmental levels of arsenic. Human umbilical vein endothelial cells (HUVEC) were exposed to 0.75 and 7.5 ppb sodium arsenite for 14 days followed by a 30 minute challenge with 20 ng/ml TNFα. Whole cell lysates were collected and western blot analysis was performed using antibodies to phospho-eIF2α (Ser51) and eIF2α. Nuclear lysates were collected and western blot analysis was performed using antibodies to phospho-ERK1/2 (Thr202/Tyr204) and ERK1/2. The results were quantified using ImageJ and normalized to total protein levels. The data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey’s multiple comparison test. *p < 0.05 compared to control cells. **p < 0.01 compared to control cells.

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Inorganic arsenic is a natural drinking water contaminant worldwide. Recently, we showed that chronic arsenic exposure exacerbates atherosclerosis and lesion inflammatory infiltration in mice. However, toxicological mechanisms of arsenic are not clearly understood. In this study, we show that sodium arsenite (0-25 μM) and its active metabolite, monomethyl arsenous acid (MMAIII; 0.5-3 mM) increased the expression of TNF-α, IL-1β, and IL-6 in murine bone marrow derived macrophages (BMM) by 2.5-60 fold (p<0.01) and the expression of IL-8 by 5-8 fold (p<0.01) in vascular endothelial cells (EC). Sodium arsenite also increased the expression of ET-1 regulated chaperones - GRP78 (1.4-fold), GRP94 (1.4-fold) and HSP70 (5.4-fold) in EC. Treatment with arsenic induced the phosphorylation of eIF2α in EC and BMM in a concentration-dependent manner. Exposure of EC to arsenic also increased the expression of activating transcription factor (ATF) 3, and ATF4 by 12-290-fold (p<0.01) and pro-apoptotic factor CHOP by 7.5-36-fold (p<0.01) respectively. Similar effects were observed in BMM. Arsenic also induced splicing of BZIP transcription factor XBP-1. Treatment with the chemical chaperone phenyl-butyric acid significantly inhibited the arsenic-induced expression of IL-8 mRNA (by 50 %) in EC. Adenoviral transfection with ATF6 increased the expression of several ER chaperone genes and efficiently inhibited (570 %) arsenic-induced IL-8 production. These data indicate that arsenic is a potent trigger of the unfolded protein response in endothelial cells and macrophages and that increased cytokine production in arsenic-treated cells is mediated, in part, by ER stress. The ER may be an important target of arsenic and the treatment with chemical chaperones may be a potential strategy for the diminishing of cardiovascular disease risk in exposed populations.

PS 960 ROLE OF ENDOPLASMIC RETICULUM STRESS IN INFLAMMATORY RESPONSES TO ARSENIC IN ENDOTHELIAL CELLS AND MACROPHAGES.

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The role of the endoplasmic reticulum (ER) in the regulation of inflammatory responses is currently under investigation. Recent studies suggest that ER stress plays a significant role in the pathogenesis of chronic inflammatory diseases. In this study, we investigated the role of ER stress in the inflammatory response of endothelial cells and macrophages to arsenic exposure. We used the chemical chaperone phenyl-butyric acid (PBA) to assess the impact of ER stress on the inflammatory response. Our data showed that PBA significantly inhibited the expression of pro-inflammatory cytokines, such as TNFα, IL-1β, and IL-6, in endothelial and macrophage cells in response to arsenic exposure. Furthermore, PBA also decreased the production of reactive oxygen species (ROS) in these cells. These findings suggest that ER stress contributes to the inflammatory response of endothelial and macrophage cells to arsenic exposure and provide evidence of a potential role for ER stress in the regulation of inflammatory responses.

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Obesity is the single most important risk factor for the development of insulin resistance and type 2 diabetes. However, chronic exposures to inorganic arsenic (iAs) have also been associated with an increased prevalence of diabetes mellitus. The current study examined the diabetogenic effects of exposures to iAs combined with consumption of a high-fat diet (HFD). Here, weaning male, C57BL/6 mice were divided into 6 groups which drank deionized water containing arsenite (iAsIII) (25 or 50 ppm) or water without iAsIII, ad libitum, for 20 weeks while consuming either a HFD (58% fat) or a low fat diet (LFD) (11% fat), also ad libitum. Body weight, adiposity, food and water consumption were monitored throughout the study. At 20 weeks fasting blood samples were collected and oral glucose tolerance tests were administered to all mice. In general, the 25 and 50 ppm groups consumed less water than control mice. iAsIII intake was estimated at 57 μg/d for 25 ppm groups and 81 μg/d for 50 ppm groups. In general, HFD groups gained significantly more fat mass and had higher fasting glucose and serum insulin levels than did their respective LFD groups. However, these measures decreased with iAsIII intake in a dose dependent manner. Oral glucose tolerance tests showed an improvement of glucose tolerance for HFD groups compared to their respective LFD groups. The degree of glucose intolerance increased with iAsIII intake in a dose dependent manner in spite of a significant decrease in adiposity, fasting glucose and fasting insulin levels. These data suggest that the diabetogenic effects of iAsIII are independent of the mechanisms traditionally associated with consumption of high fat diets and/or obesity in mice.

PS 964 EFFECT OF ARSENIC ON MOUSE EMBRYONIC STEM CELL GENE EXPRESSION FOR DIFFERENTIATION.

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Mouse embryonic stem (mES) cells (ES-D3; ATCC) are pluripotent cells derived from the inner cell mass of mouse blastocysts and differentiate into the 3 germ layers. We examined the effect of arsenic (As; 0.054 mmol/l) on tight junction (TJ)
Carcinogenicity of Whole Life Exposure to Inorganic Arsenic in Mice.

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Inorganic arsenic is a multi-site human carcinogen. We previously developed a mouse model where inorganic arsenic exposure \textit{in utero} either acts as a complete carcinogen or enhances carcinogenic response to other agents, producing tumors at multiple sites in adult offspring. However, this model does not reproduce human environmental arsenic exposure, which occurs during the whole life (WL). Thus, we studied the effects of WL inorganic arsenic exposure on tumor outcome in CD1 mice. Mice were exposed to 0, 6, 12 or 24 ppm arsenic (as sodium arsenite) in the drinking water 2 weeks prior to and through breeding (males then discarded), during pregnancy, lactation (dams discarded), after weaning and through adulthood for up to 2 years and tumors were assessed in these adults (initial n = 30). No reductions in body weight occurred. Dose-related increases in lung adenocarcinoma occurred in females (0 ppm 7%, 6 ppm 21%, 12 ppm 28%, 24 ppm 43%) and males (0 ppm 10%, 6 ppm 28%, 12 ppm 32%, 24 ppm 39%). Liver tumors (mostly hepatocellular carcinoma) showed dose-related increases in males (0 ppm 19%, 6 ppm 36%, 12 ppm 40%, 24 ppm 57%) and females (0 ppm 3%, 6 ppm 15%, 12 ppm 15%, 24 ppm 18%). Uterine tumors (primarily carcinoma) showed dose-related increases to a 32% maximum (24 ppm) over control (3%). Dose-related increases in ovarian tumors (mostly carcinoma) occurred and were significantly elevated over control (0%) even at the lowest dose (6 ppm, 17%) and maximal at 24 ppm (32%). Adrenal tumors were increased at all arsenic doses in males and females. Arsenic induced gall bladder tumors in males and urinary bladder hyperplasia in both sexes. Although significantly lower doses were used, the target sites in this work are very similar to our prior transplacental studies and together these studies indicate events \textit{in utero} likely "initiate" tumors while other arsenic exposures enhance tumor incidence. These data indicate WL inorganic arsenic exposure can induce tumors in mice at levels as low as 6 ppm in the drinking water.

Evaluation of Oxidative Stress by Inorganic Arsenic and Oral Contraceptive Pill Among the Females in Arsenic-Contaminated Area of Bangladesh.

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Background and objectives: Toxicity of arsenic has been shown to exhibit gender-related difference, while some inconsistency exist among the reports. To address this issue, we have compared the oxidative stress markers among the arsenic-exposed women with or without using estrogenic pills. Design and participants: Two oxidative stress markers, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-isoprostane in spot urine samples, were evaluated in estrogenic contraceptive pill users and non-users living in two Bangladesh communities. Samples were obtained from 228 healthy women, aged 18–40 years, from two communities (15 women in an "exposed" and 113 in a "control" community). Approximately half of the participants were contraceptive pill user, who used estrogenic pills more than one year, while those who never used the pills comprised the other half. Measurements: Urinary concentrations of arsenic (UA) and selenium (DRC-ICP-MS), 8-OHdG (by HPLC-ECD), and F2 isoprostanes (by ELISA) were measured. Speciation of urinary arsenic was determined using HPLC-ICP-MS. Arsenic in the drinking water (tube well water) was also determined by ICP-MS. Results: As expected, UA was significantly correlated with tube well arsenic, and UA arsenic and oxidative stress markers were significantly higher in the exposed group than control group. UA was highly significantly correlated with 8-OHdG (r=0.40) and with 8-isoprostane (r=0.32). Both oxidative indices as well as UAs were significantly lower in pill users than non-users in both As-exposed and control subjects; in multiple regression analyses, use of estrogenic pills exerted significant, lowering effects on the two oxidative indices as well as on UAs. Conclusion: The estrogenic pill use may exert protective effect on arsenic toxicity, which may be related with the reported gender-related difference in dermatological toxicity of the metalloid.

Arsenic Transformation/Adaptation Predisposes Human Skin Keratinocytes to Oxidative DNA Damage Yet Enhances Their Survival.

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Inorganic arsenic and ultraviolet (UV) irradiation, known human skin carcinogens, may act synergistically in skin carcinogenesis. We have developed a skin cancer model using human skin keratinocytes (HaCaT cells) malignantly transformed by low-level arsenite (100 nM, 50 weeks; termed As-TM cells) that are also fully adapted to arsenic toxicity. Oxidative DNA damage (ODD) is a possible mechanism in arsenic carcinogenesis and a hallmark of UV-induced skin cancer. Prior work indicated As-TM cells may acquire resistance to UV-induced apoptosis but not UV-induced ODD, though the method used for measuring ODD was highly prone to significant artifacts. In this study As-TM cells were acutely irradiated with UV (UVA, 25 J/cm²), incubated 18 hrs further and apoptosis (by flow cytometry), ODD (by the immuno-spin trapping method) and gene expression (by RT-PCR) were assessed. ODD was not observed over the 30-week arsenic-induced malignant transformation period, likely because HaCaT cells poorly methylate arsenic. Although UV markedly increased ODD in control, it was further increased by over 50% in As-TM cells. As-TM cells were resistant to UV-induced apoptosis despite this enhanced ODD. Exposure of As-TM cells to the direct oxidant H₂O₂ (1 mM) for 24 hrs showed they were resistant to H₂O₂-induced apoptosis despite increased ODD. The response of various apoptotic factors and oxidative stress genes were reduced in As-TM cells after UV exposure, including reduced Bcl2/Bax ratio and caspase 3 expression, as well as reduced expression of Nf2 and Keap1. Several Nf2 related genes (HO-1, GCLC, GCLM, SOD1 and SOD2) showed a markedly diminished response in As-TM cells treated with UV compared to control. Thus, the potential synergy with UV and arsenic in skin cancer could be due to an adaptation to chronic arsenic exposure, which mitigates the oxidative stress response, but allows apoptotic by-pass and provides for enhanced survival even in the face of increased UV-induced oxidative stress and increased ODD.
Arsenic trisulfide is used to treat certain leukemias and has potential for treatment of solid tumors, but hepatotoxicity is a major limiting side effect. O-vinyl 1,2-(carboxylato)pyrroolidin-1-yl)diazene-1-ium-1,2-diolate (V-PROLI/NO) is a nitric oxide donor prodrug that is metabolized by liver cytochromes P450 to release NO. The effects of V-PROLI/NO pretreatment on the toxicity of arsenic (as NaAsO₂) were studied in vitro in a rat liver (TRL 1215) cell line. These cells acted upon the produg to release NO, as assessed by nitrite levels, in a concentration- and time-dependent fashion to maximal levels of 40-fold above background. In cells pretreated with V-PROLI/NO (200 μM, 24 h) then exposed to arsenic for additional 24 h, arsenic was much less toxic (LC₅₀ = 65.8 μM) than in control cells (LC₅₀ = 18.4μM). The reduced cytotoxicity was related to the level of NO produced. V-PROLI/NO increased Cyp1a1, Gpi1, and HO-1 transcriptional expression. Indeed, increased Cyp1a1 transcript was directly related to NO production. Similarly, the increases in Cyp1a1 transcript stimulated by V-PROLI/NO were directly correlated to increased arsenic LC₅₀. Increased GST-P is important in arsenic efflux while HO-1 is important in adaptation to arsenic-induced oxidative stress. V-PROLI/NO pretreatment markedly reduced arsenic-induced apoptosis, as measured by DNA fragmentation, and suppressed phosphorylation of JNK1/2, a key event in apoptosis. Arsenic alone increased metallothionein (MT), a metal-binding protein important in arsenic tolerance, while V-PROLI/NO pre-treatment before arsenic caused additional increases in MT levels. Thus, V-PROLI/NO protects against arsenic toxicity in liver cells, reducing cytotoxicity, apoptosis and blocking dysregulation of MAPKs. This appears to be through generation of NO formed after metabolism by the liver cell enzymes, possibly including Cyp1a1.

Arsenic in drinking water is a problem in many developing countries such as Taiwan and Bangladesh. Currently, no oral binding agent has been clearly demonstrated to be successful for the mitigation of arsenic toxicity. Ferrihydrite, an iron oxy-hydroxide, was recently tested in vitro for its ability to serve as an enterosorbent for arsenic found in drinking water. The goals of this research were to evaluate the ability of an industrially produced ferrihydrite (IPF) to sorb arsenic in a series of in vitro experiments and validate its safety when included in the diet of rats. IPF was tested for its ability to bind arsenic as sodium arsenite, As(III) and sodium arsenate, As(V) in binding isotherms and in a simulated gastro-intestinal (GI) model. IPF also tested for its ability to protect Hydra from toxicity. IPF was included in the diet of 4-week old male Sprague-Dawley rats at 0.5% for 2 weeks, and subsequently, serum was tested for iron levels and serum biochemistry. In the in vitro experiments, IPF was found to strongly sorb both As(III) and As(V) with a Qmax of 0.452 mol/kg and 0.252 mol/kg, respectively. IPF also successfully removed As in a simulated GI model exhibited by Qmax values of 0.246 mol/kg and 0.344 mol/kg for As(III) and 0.311 mol/kg and 0.365 mol/kg for As(V) in the simulated stomach and intestine, respectively. IPF protected Hydra at levels up to 200 and 2.5 times the minimal affective concentration for As(III) and As(V), respectively. Rats eating 0.5% IPF in their diet showed no significant differences in serum iron levels, serum biochemistry, serum vitamin A and E, or feed conversion rates. These experiments demonstrated IPF’s ability to sorb both As(III) and As(V) at a high capacity, and show that it protects a simple aquatic organism from As toxicity. IPF at 0.5% was shown to be apparently safe and was well tolerated by rats. Work is ongoing to verify IPF’s efficacy in a rodent model.

Inorganic arsenic (iAs), a common drinking water contaminant, is one of the environmental carcinogens with a suspected epigenetic mode of action. Recent studies using a mouse transplacental model for iAs carcinogenesis showed that exposures of pregnant C571 mice to iAs in drinking water result in an increased incidence of liver tumors in adult male offsprings. S-adenosylmethionine (SAM) supplies methyl groups for both the maintenance of normal DNA methylation pattern and the enzymatic methylation of iAs. Thus, it is possible that the mechanisms underlying the transplacental iAs carcinogenesis involve changes in the DNA methylation pattern in the fetal liver due to competition between DNA methyltransferases and iAs-methyltransfer for SAM. Results of our preliminary studies show that in utero exposure to iAs significantly changes the genomic DNA methylation pattern in livers of mouse fetuses and that the most affected genes and gene networks are associated with one carbon metabolism, inflammation, and cancer. Expression of genes involved in one-carbon metabolism and DNA methylation is increased in livers of fetuses exposed to iAs as compared to untreated controls. In addition, in utero exposure to iAs increases significantly SAM concentration in fetal livers in a manner that is consistent with a compensatory response to an increased demand for methyl groups. These results support the epigenetic mechanism for the transplacental iAs carcinogenesis and provide basis for future studies to examine the role of dietary precursors of SAM in modulation of this mechanism.
THE POSSIBLE PROTECTIVE ROLE OF CALBINDIN-D28K, A CALCIUM BINDING PROTEIN, IN RESISTANCE TO METHYLMERCURY TOXICITY IN RAT PC12 CELLS.

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Calbindin-D28K is a “buffer type” protein which binds Ca2+ and is associated with resistance to Ca2+ mediated cell death. MeHg is an environmental contaminant, that disrupts [Ca2+], homeostasis and causes selective cytotoxicity to cerebellar granule cell neurons while adjacent Purkinje cells remain viable. One reason for this may be that Purkinje cells express CB while granule cells do not. To investigate the role of CB in resistance to MeHg toxicity, rat pheochromocytoma PC12 and HEK293 cells were transiently transfected with a CB expression cDNA clone. The CB expressing cells were then perfused with 1 and 5μM MeHg and changes in intracellular Ca2+ were measured using single cell microfluorimetry and fura-2. As described previously, MeHg induces a biphasic increase in fura-2 fluorescence. Expression of CB in PC12 cells delayed the time to onset of the increase in fura-2 fluorescence induced by 1 and 5μM MeHg, as compared to set cells. At 1μM MeHg, PC12-set mean time to onset to phase 1 was 10.6 min while PC12-CB was 15.8 min. The mean time to onset for phase 2 of PC12-set and CB cells were 14.4 min and 20.0 min respectively. At 5μM MeHg, PC12-set and CB cell mean time to onset were 18 min and 3.4 min respectively. In contrast to the PC12 cells, HEK-CB cells only demonstrated a delayed time to onset at 1μM when compared to set. CB may play a protective role at low concentrations but at higher concentrations of MeHg CB can no longer protect the cell from excitotoxicity. The expression of CB alone is not the only reason that Purkinje cells exhibit a greater resistance to MeHg toxicity than do granule cells. Supported by NIH grant R01-ES05299.

TOXIC EFFECTS OF MANGANESE ON MITOCHONDRIAL CATALASE AND CYTOCHROME C OXIDASE IN GILL OF CRASSOSTREA VIRGINICA.


Manganese (Mn) is an essential trace nutrient in humans. Overexposure can cause Manganese, a Parkinson’s-like disorder. The mechanism of toxicity remains largely unknown. Mn accumulates in mitochondria. It cause dysfunction disrupting en-
We showed Mn disrupts the dopaminergic mechanism in people. Previously, we showed lateral cilia of gill of C. virginica are innervated by serotonin (HT) nerves. DA slows down ciliary and serotonin accelerates them. Lateral cilia of gill of C. virginica are innervated by serotonin (HT) and dopamine (DA). They increase or decrease beating, respectively. This has been well studied in bivalves, but the fast movement of cilia has prevented microelectrode studies of membrane potentials in the ciliated cells other than the elegant studies of Murakami and Takahashi in the 1970s. Voltage sensitive fluorescent probes like DiBAC have been developed that change fluorescent intensity in relation to membrane potential. We observed membrane potentials of lateral cells of C. virginica while measuring beating rates. HT or 5 Hz electrical stimulation (ES) to the branchial nerve which innervates gill caused prolonged membrane depolarizations similar to plateau potentials, and increased ciliary beating. DA or 20 Hz ES to the nerve after exciting cilia, repolarized the membrane and decreased beating. Manganese (Mn) is a neurotoxin causing Manganism, a Parkinsons-like disorder. Lateral cell cilia of gill of C. virginica are innervated by dopamine (DA) and serotonin (HT) nerves. DA slows down ciliary and serotonin accelerates them. High levels of manganese (Mn) are neurotoxic to people, causing Manganism, a Parkinsons-like disease. Recently, p-aminosalicylic acid (PAS) was reported as an effective treatment of severe Manganism. Previously we showed short-term treatments of C. virginica with Mn disrupts the DA innervation of gill. The impairment was decreased by co-treating with PAS. We used a pharmacological and immunofluorescence study of DA receptors in this system to learn which receptor type was being effected by Mn. DA receptors are classified as D1-like and D2-like, with each subtype. Beating of cilia were measured by stroboscopic microscopy; D1 and D2 agonists and antagonists were applied to gill to determine their efficacy in altering beating. D2 agonists were effective in mimicking DA, and D2 antagonists were effective in blocking the actions of DA. D1 agonist and antagonist did not alter the beating of the cilia nor block the effects of DA. We further examined the receptor by using a primary antibody against D2 receptors followed by an FITC linked secondary antibody to visualize them. Gill were exposed to antibodies and prepared for light microscopy. Control sections without primary antibody exposure were similarly treated and viewed. Antibody treated sections showed bright FITC fluorescence in the lateral ciliated cells and other areas of gill. Control sections did not. The study shows the postsynaptic DA receptors involved in the cilio-inhibitory response of the lateral cells of gill are D2 type. The study shows this preparation is a good model for pharmacological studies of DA function as well as the pharmacology of drugs affecting biogenic amines. This work was supported by grants 2R25GM0600305 of NIGMS, 0516041071 of NYSDOE, 0622197 of NSF and P382A080040 of the USDE.

Manganese (Mn) is an essential metal that at excessive levels in brain produces Manganism, which is similar to Parkinsons disease. The mechanism toxicity is not completely understood but may be related to oxidative stress and damage to the dopaminergic system in people. Previously, we showed lateral cilia of gill of C. virginica are controlled by serotonin-dopaminergic innervations and Mn disrupts the cilio-inhibitory dopaminergic system. To study effects of Mn on mitochondria, a potential target for cellular damage due to oxidative stress, we prepared mitochondria from the gill of C. virginica and used a YSI Micro-Biological Oxygen Monitor with micro-batch chambers. Mn (5µM - 5 mM) caused a decrease in mitochondrial O2 consumption which was blocked by pretreating with 1 mM of calcium disodium EDTA (EDTAca) or p-Aminosalicylic Acid (PAS), an anti-inflammatory agent which may have chelating abilities. Both drugs are being tested as therapeutic agents for Manganism. Adding EDTAca or PAS to Mn treated mitochondria partially reversed the effects of Mn. We also studied effects of Mn on mitochondrial membrane potentials using the anionic oxonol-based membrane potential-sensitive fluorescent dye, HLβ021-152, which increases its fluorescent intensity upon membrane depolarization. Mn decreased the mitochondrial membrane potential. This was partially blocked by co-treatment with PAS. The study shows Mn reduces O2 consumption and causes depolarization of mitochondrial membrane potentials. The chelator EDTAca effectively blocked the effects of Mn and may be beneficial in reversing toxic effects of Mn accumulations. Our results suggest the ability of PAS to ameliorate symptoms of Manganism might be related to chelation. The work was supported by grants 2R25GM0600305 of NIGMS, 0516041071 of NYSDOE, 0622197 of NSF and P382A080040 of the USDE.
维持大脑铜稳态对大脑功能健康至关重要。通过系统性Fe缺乏（FeD）或过载（FeO）的动物模型，发现铜蛋白的表达水平会显著变化。具体而言，FeD组和FeO组的大鼠肝脏中铜蛋白的表达显著增加，而FeO组的大鼠肝脏中铜蛋白的表达显著降低。这些结果表明，铜蛋白的表达可能受Fe水平的影响，而FeD和FeO组的大鼠肝脏中铜蛋白的表达变化情况不同。

Previous studies from our laboratory have shown that exposure to lead (Pb) not only causes Pb accumulation but also increases the retention of beta-amyloid (Aβ) in the choroidal plexus (CP), a brain tissue where the blood-cerebrospinal fluid barrier (BBB) regulates Cu fluxes between the blood and the brain. The blood-brain barrier (BBB) regulates Cu fluxes between the blood and the brain. However, whether Pb may affect P-gp in the CP and therefore alter Aβ transport by BCB is located. Literature also suggests that P-glycoprotein (P-gp) is responsible for the transport of Aβ in the brain parenchyma and choroid plexus to any extent, the FeD animals showed significant increases in Cu levels in both the brain and choroid plexus. ICP-MS analysis revealed that while serum Cu was linearly associated with CSF Cu (r=0.797, p<0.01), there was no association between CSF and serum Fe. Serum Cu levels were inversely associated with that of Fe (r=-0.745, p<0.01); yet no association between Pb and Cu was observed in the CSF. Furthermore, the expression of DMT1 and Cu influx pathway proteins (ATP8A, ATP7A, and ATOX1) leading to an altered Cu level in the CSF and brain parenchyma. (Supported by NIH/NIEHS ES-008146)

984 INCREASED P-GLYCOPROTEIN EXPRESSION AT THE BLOOD-CEREBROSPLINAL FLUID BARRIER FOLLOWING ACUTE LEAD EXPOSURE.

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Previous studies from our laboratory have shown that exposure to lead (Pb) not only causes Pb accumulation but also increases the retention of beta-amyloid (Aβ) in the choroidal plexus (CP), a brain tissue where the blood-cerebrospinal fluid barrier (BBB) is located. Literature also suggests that P-glycoprotein (P-gp) is responsible for the transport of Aβ in the brain parenchyma and choroid plexus to any extent, the FeD animals showed significant increases in Cu levels in both the brain and choroid plexus. ICP-MS analysis revealed that while serum Cu was linearly associated with CSF Cu (r=0.797, p<0.01), there was no association between CSF and serum Fe. Serum Cu levels were inversely associated with that of Fe (r=-0.745, p<0.01); yet no association between Pb and Cu was observed in the CSF. Furthermore, the expression of DMT1 and Cu influx pathway proteins (ATP8A, ATP7A, and ATOX1) leading to an altered Cu level in the CSF and brain parenchyma. (Supported by NIH/NIEHS ES-008146)

985 GENDER INFLUENCE ON ABILITY OF ORAL MANGANESE TO DAMAGE BRAIN.

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Changes in the levels of gliarial fibrillary acidic protein (GFAP) have been argued to be a biomarker for neurotoxicity. The potential of manganese sulfate (MnSO4), given daily by gavage for either 14 or 90 days, to alter the GFAP, was studied in rats. Male and female rats were randomly given by gavage one of six liquid mixtures (sus-
treated with mercury and then with NEM. Following recovery of the enzyme with dihithiothreitol or cysteine, we found that mercury prevented inactivation of SPR by NEM. These data indicate that mercury targets Cys171 in the enzyme. Taken together our data demonstrate that SPR is an important target for mercury toxicity. Inhibition of SPR can lead to cellular depletion of tetrahydrobiopterin, a process that inhibits enzymes dependent on this cofactor. Supported by CA100994, CA132624, CA093798, ES004738, ES005022, GM034310 and AR055073.

Inhibition of SPR can lead to cellular depletion of tetrahydrobiopterin, a process that inhibits enzymes dependent on this cofactor. Supported by CA100994, CA132624, CA093798, ES004738, ES005022, GM034310 and AR055073.

988 NEUROTOXIC ACTIONS OF 6-OHDA, 5, 7-DHT AND MANGANESE ON SEROTONERGIC AND DOPAMINERGIC INNERRATION OF LATERAL CILIATED CELLS OF GILL OF CRASSOSTREA VIRGINICA.


Cilia of lateral cells of gill of *Crassostrea virginica* are innervated by serotonin (HT) and dopamine (DA) which increase or decrease beating rates, respectively. 5,7-Dihydroxytryptamine (5,7-DHT) is a neurotoxin destroying HT neurons. 6-Hydroxydopamine (6-OHDA) destroys DA neurons. Manganese (Mn) causes Manganism. Mn affects dopaminergic systems but its mechanism is unclear. Our studies show Mn disrupts dopaminergic innervation of lateral ciliated cells of gill of *C. virginica*. This study contrasts actions of 6-OHDA, 5,7-DHT and Mn with physical denervation of the branchial nerve on ciliary activity. Animals were treated 3-7 days with 6-OHDA, 5,7-DHT or Mn. Control animals were vehicle treated. Beating was measured by strobescopic microscopy in response to superfusion of DA and HT, and electrical stimulations (ES) of the branchial nerve. 6-OHDA caused supersensitivity to DA, an inability of inhibitory ES of the branchial nerve to slow down cilia, but no impairment to the dopaminergic innervation. Mn caused an inability of DA and inhibitory ES of the branchial nerve to slow down cilia, and no impairment of the serotonergic innervation. Physical denervations caused supersensitivity to DA after 5 days. This study shows the 3 neurotoxins have different mechanisms of action. This can be helpful in designing potential therapeutic treatments of neurological disorders. This work also shows the preparation is a useful model to study regulatory mechanisms of ciliary activity as well as the pharmacology of drugs affecting biogenic amines. This work was supported by grants R29GM06003-05 of NIGMS, 0516401071 of NYSDOE, 0622197 of NSF and P382A080040 of the USDE.

989 PRETREATMENT WITH ASCORBIC ACID AMELIORATES CHANGE IN RATS REPEATEDLY EXPOSED TO LEAD.

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Chronic lead exposure has been associated with low intelligent quotient amongst individuals. Oxidative stress is one of the mechanisms implicated in lead-induced cognitive impairment. The present study examined the effects of pre-treatment with ascorbic acid on learning and memory impairments following repeated low-dose lead acetate exposure in Wistar rats. 40 young adult Wistar rats were divided into 4 groups of 10 animals in each group. Group I (control) and II were administered distilled water (2 ml/kg) and lead acetate (250 mg/kg - 1/20th LD50 of 5000 mg/kg determined in an earlier study), respectively. Group III were administered 100 mg/kg of ascorbic acid, while rats in group IV were pre-treated with ascorbic acid (100 mg/kg) followed by lead acetate (250 mg/kg) 30 minutes later. The regimens were administered orally once daily for 6 weeks. Learning was examined 48 hours to the termination of the study, while short-term memory was evaluated 24 hours later using the step-down avoidance inhibitory learning task apparatus. Five animals from each group were sacrificed and the brain evaluated for maldonaldahyde (MDA) concentration, as an index of lipid peroxidation. The results showed that rats exposed to lead acetate demonstrated poor learning acquisition and short memory retention compared to the control and ascorbic acid-pretreated groups. An increase in MDA concentration was observed in the lead-treated animals when compared with those in the other groups. In conclusion, ascorbic acid exerted protective effects on cognitive changes induced in Wistar rats repeatedly exposed to low dose of lead partly due to its antioxidant effect.

990 MANGANESE NEUROTOXICITY IS ASSOCIATED WITH PROTEASOME DYSFUNCTION IN α-SYNuclein OVER EXPRESSED DOPAMINERGIC NEURONS.

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Manganese (Mn) induces neurological disorders similar to Parkinson's disease. Workers exposed to Mn from dust and fumes, welding, thermal cutting and boiler maintenance may be at risk of Mn poisoning. Impairment of the ubiquitin-proteasome degradation system has recently been suggested to be related to the onset of neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. We have previously shown that α-synuclein over expressed neuronal cells enhanced Mn-induced neurotoxicity and are associated with oxidative stress. Here, we examined whether Mn exposure impairs proteasomal function and subsequently promotes apoptosis in dopaminergic cells over expressed with human α-synuclein. The proteasomal enzymatic activity was measured by a fluorometric method. Selective oxidative stress parameters including malondialdehyde (TBARS) were measured in α-synuclein over expressed cells following Mn exposure. Cell survival and apoptosis were examined through MTT and terminal transferase-mediated dUTP nick end-labeling, caspase-3 activity and DNA fragmentation assays. Increased expression of α-synuclein significantly reduced proteasomal activity with an enhanced reduction after Mn treatment. The severity of impairment was proportional to neuronal cell death. In addition, proteasomal inhibition preceded cell death after Mn treatment and α-synuclein over expressed cells were more sensitive to Mn toxicity. Measurement of caspase-3 activity and DNA fragmentation confirmed the enhanced sensitivity of α-synuclein over expressed cells. However, the inhibition of proteasomal activity and cell death were reversed by antioxidant N-acetylcysteine pretreatment indicating that proteasomal dysfunction may be associated with Mn-induced neurotoxicity, which may be connected with oxidative damage. These findings suggest that increased expression of α-synuclein may contribute to the molecular pathogenesis of Mn-induced neurotoxicity.

991 THE EFFECT OF TUNGSTEN ALLOY SURROGATES ON PC12 CELL CYTOXICITY AND NEUROTGENESIS.

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The neurotoxicity of metals such as tungsten (W), nickel (Ni), and cobalt (Co) has not been examined in detail. Moreover, the combined effect (whether additive, synergistic or neither) of these metals in a mixture is even less understood. Prior data indicate that deposition of radiolabeled tungstate (Na2WO4) in the brain is not accomplished through direct transport mechanisms along the olfactory nerves; however, investigators have reported that tungsten can be detected in rat brain following intraventricular or intraperitoneal injection and oral gavage. We explored the neurotoxicity of tungsten alloy containing W, Ni and Co in the PC12 cell line in vitro model. The PC12 cell model allowed for direct testing the neurotoxicity of the metal alone or as an alloy. The metal transport across the blood brain barrier and the toxicity of tungstate alone is less toxic to PC12 cells than the alloy surrogate. As a first step, the soluble metal profile of W/Ni/Co alloy (3 pellets-20mg/pellet) exposed to aqueous solutions was quantified using Inductively Coupled Plasma – Mass Spectrometry. The ratios of mobilized alloy metals in the profile were used in the in vitro exposure experiments. The dose response of PC12 cells to the individual metal ions, metal ion combinations (W/Ni, W/Co, Co/Ni) and alloy surrogate was determined by measuring cytotoxicity, proliferation and neurotogenesis. Our preliminary findings suggest that metal solubilization is partially dependent on the solvent and that the relative ratio of W/Ni/Co impacts PC12 cytoxicity. Microarray analysis is currently underway to assess tungstate versus alloy specific changes in PC12 cell gene expression.

992 DIFFERENTIAL EFFECTS OF MANGANESE ON LPS INDUCTION OF HO-1 IN MICROGLIA AND NEURONAL CELL LINES.

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In humans, excess exposure to manganese (Mn) is neurotoxic; it causes a parkinsonian type disorder (manganism). Several studies provide evidence for the role of glial-derived (microglia and astrocytes) inflammatory products in Mn neurotoxicity. Inducible heme oxygenase (HO-1), which is responsible for the cleavage of

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Manganese (Mn) is an essential trace element necessary for a variety of physiological processes. Despite its essentiality, Mn in excess leads to neurotoxicity. The neurotoxicity observed from Mn accumulation in the brain has been associated with dysfunction of the basal ganglia and alterations in metal ion homeostasis. Our lab has reported a gene-environment interaction between polyglutamine-expanded huntingtin (HTT) protein and the neurotoxicant metal ion, Mn. We find that mutant htt suppresses Mn toxicity and impairs Mn accumulation. Huntington disease (HD) results in selective degeneration of medium spiny neurons (MSNs) of the corpus striatum. Interestingly, the striatum is also a major target of Mn accumulation in the brain. To evaluate the mechanism by which mutant HTT alters Mn accumulation, we have developed a novel assay to assess Mn uptake, efflux and cellular storage. This fluorometric high-throughput assay takes advantage of the metal binding fluorescent dye, Fura-2 that exhibits selective quenching properties in the presence of Mn at the isobestic point (360nm) of calcium. This high-throughput assay utilizes changes in Fura-2 fluorescence as an indirect measure of total and compartmentalized Mn content. The assay provides a means to measure Mn transport kinetics and storage features to those of neurons. Cell death in the entire animal was assessed using the live/dead dye SYTOX Orange. Synchronized cultures of L1 larvae were exposed to 0.1-10.0 mM CuSO4 in 2X K medium, then treated with SYTOX Orange. L1s were mounted on agarose pads and viewed by fluorescence microscopy. Previous studies in the nematode Caenorhabditis elegans (Williams et al. Proc Clin Biol Res 253:163-70, 1987; Toxicol Ind Health 6:325-40, 1990) concluded that copper is not a neurotoxin because the behavioral EC50 for Cu is much higher than the EC50 for neuronal damage. The observation that neurons are much more sensitive to copper-induced damage than somatic cells demonstrates that copper is neurotoxic to C. elegans. Our findings that paralysis does not reliably indicate death. The purpose of this study was to reassess if neurons of C. elegans are more vulnerable to copper toxicity than the highly metabolically active excretory cell, or the animal as a whole. In this study, copper-induced anatomical damage to specific cells was quantified in strains of C. elegans where green fluorescent protein (GFP) was expressed in specific neurons or the excretory cell. The excretory cell is a somatic cell with similar anatomical and metabolic features to those of neurons. Cell death in the entire animal was assessed using the live/dead dye SYTOX Orange. SYTOX Orange-stained cultures of L1 larvae were exposed to 0.1-10.0 mM CuSO4 in 2X K medium, then treated with SYTOX Orange. L1s were mounted on agarose pads and viewed by fluorescence microscopy. Examination of L1s with GFP-tagged neurons exposed to CuSO4 for at least 6 hours showed time- and concentration-dependent damage (blebbing or broken processes) compared to controls. All animals treated for 12 hr at concentrations of 1.0 mM or more had damaged neurons. The excretory cell resisted copper-induced damage, which demonstrates a selective effect of Cu+ upon neurons. Though most copper-treated L1s were nonresponsive, SYTOX staining showed that actual death (majority of cells stained) was rare up to 10 mM CuSO4 for 12 hr; thus, the LC50 is much higher than the EC50 for neuronal damage. The observation that neurons are more susceptible to copper-induced damage than somatic cells demonstrates that copper is neurotoxic to C. elegans. Our findings that paralysis does not reliably indicate death suggest that C. elegans researchers should not depend on paralysis as the sole criterion for death.
**997 IN VIVO CORRELATES OF THE MANGANESE ACCUMULATION DEFICIT IN A HUNTINGTON DISEASE MOUSE MODEL.**

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Huntington disease (HD), an inherited neurodegenerative disorder principally affecting the striatum, is caused by expansion of a polymultimeric tract within the Huntingtin (HTT) protein. While severity is correlated with repeat length, it is also strongly influenced by unidentified environmental factors. We have reported an unexpected gene-environment interaction wherein expression of mutant HTT is associated with neuroprotection against manganese (Mn) toxicity. We hypothesize that mutant HTT may impair Mn accumulation and alter metabolic and protein markers of Mn toxicity. Using inductively coupled plasma mass spectrometry we find that Mn-exposed mice expressing mutant HTT (YAC128Q) have decreased striatal (but not cortical) Mn accumulation compared to wild-type littermates. Furthermore, this phenotype does not correlate with changes in the expression of Mn transporters such as transferrin receptor, DMT1 and MTPI. To identify pathophysiological correlates of the Mn accumulation deficit we analyzed striatal extracts for selected metabolites and performed proteomic analysis following a one-week Mn exposure paradigm. In the absence of any significant changes in analyzed amino acids and neurotransmitters, principal component analysis of proteomics data revealed that a segregation of wild-type versus mutant striatal proteomes by the major principal component with vehicle exposed mice was absent in Mn-exposed animals. Finally, Mn clearance experiments have shown that 3 weeks post-exposure, Mn levels are still elevated in wild-type striatum, but are restored to basal levels in YAC128Q striatum. We conclude that expression of mutant HTT impairs uptake, storage or efflux of Mn in the striatum, and that this interaction may suppress mutant HTT-dependent changes in the proteome. Understanding the mechanism by which mutant HTT suppresses Mn accumulation may reveal neuroprotective strategies for Mn toxicity and provide insight into the pathophysiology of HD.

**998 SUPPRESSION OF MANGANESE-INDUCED OXIDATIVE DAMAGE AND NEURONAL INJURY.**

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Exposure to excessive manganese (Mn) levels leads to neurotoxicity (manganese) resembling Parkinson’s disease. The mechanisms by which Mn induces neuronal cell death are not well defined. In the present study, we assessed the effects of Mn on reactive oxygen species (ROS) formation, changes in high-energy phosphates (HEP) and associated neuronal dysfunctions both in vitro and in vivo. Results from our in vitro study showed significant (P<0.01) increase (143%) in biomarkers of oxidative damage, F2-isoprostanes (F2-Isop), and depletion of ATP (39%) in primary rat cortical neurons following Mn (500 μM) exposure for 2 hours. These effects were suppressed when the neurons were pretreated for 30 min with 100 μM of antioxidants, a spin trapping agent PBN (alpha phenyl-N- tert butyl nitrate), a hydrophilic vitamin E analog trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) or an anti-inflammatory agent indomethacin. Results from our in vivo study confirmed significant (p<0.05) increase in cerebral Mn and F2-Isop levels along with progressive spine degeneration and dendritic damage of striatal medium spiny neurons (MSNs) of mice exposed to Mn (100 mg/kg, sc). Additionally, pre-treatment with vitamin E (100 mg/kg, ip) or ibuprofen (140 μg/ml drinking water for two weeks) suppressed the Mn-induced increase in cerebral F2-Isop and rescued the MSNs from dendritic atrophy and loss of dendritic spines. Our findings suggest that mediation of oxidative stress/mitochondrial dysfunction and control of alterations in biomarkers of oxidative injury, neuroinflammation and synaptoden- dritic degeneration may provide a therapeutic strategy for suppression of dysfunctional dopaminergic transmission and slowing of the Mn-induced neurodegenerative process (Supported by DoD W81XWH-05-01239).

**999 FERROPORTIN IS A MANGANESE-RESPONSIVE PROTEIN THAT DECREASES MANGANESE CYTOXICITY AND ACCUMULATION.**

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Although manganese (Mn) is an essential element for human development and growth, chronic exposure to excessive Mn levels can result in psychiatric and motor disturbances, referred to as manganese. However, there are no known mecha- nisms for efflux of excess Mn from mammalian cells. Here, we test the hypothesis that the cytoplasmic iron exporter ferroportin (Fpn) may also function as a Mn exporter to attenuate Mn toxicity. Using an inducible human embryonic kidney (HEK293T) cell model, we examined the influence of Fpn expression on Mn-in- duced cytotoxicity and intracellular Mn concentrations. We found that induction of an Fpn-green fluorescent protein (GFP) fusion protein in HEK293T cells was cytoprotective against several measures of Mn toxicity including Mn-induced cell membrane leakage, Mn-induced reductions in glutamate uptake and decline in cell survival. Fpn-GFP mediated cytoprotection correlated with decreased Mn ac- cumulation following Mn exposure. Thus Fpn expression reduces Mn toxicity con- comitant with reduced Mn accumulation. To determine if mammalian cells may utilize Fpn in response to increased intracellular Mn concentrations and toxicity, we assessed endogenous Fpn levels in Mn-exposed HEK293T cells and in mouse brains in vivo. We find that Fpn expression in Mn-exposed mice is associated with a significant increase in Fpn levels. Furthermore, mice exposed to Mn showed an increase in Fpn levels in cerebellum and cortex. Collectively, these results indi- cate that (1) Mn exposure promotes Fpn protein expression, (2) Fpn expression re- duces net Mn accumulation and (3) reduces cytotoxicity associated with exposure to this metal.

**1000 GABA INCREASES IN BASAL GANGLIA IN MANGANESE EXPOSED SMELTERS.**

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Understanding the inherent vulnerability of dopaminergic, glutamatergic and GABAergic systems to manganese (Mn) should provide critical insights into the mechanisms of Mn-induced neurotoxicity. In this study we used Magnetic Resonance Spectroscopy (MRS) to determine in vivo concentrations of glutamate (Glu), γ-aminobutyric acid (GABA), N-acetylaspartate (NAA), creatine (Cr) and five other brain metabolites in the basal ganglia of a well-established cohort of 10 Mn-exposed smelters and 10 control subjects with no history of Mn exposure from an Mn-Fe alloy manufacturer in Zunyi, China. In addition to anatomical MRI, which was used to determine the pallidal index (PI; intensity ratio between the globus pallidus region and a neck muscle region in T1-weighted images), MRS was performed in five brain regions, including a volume encompassing the thalamus, globus pallidus and some putamen. A special GABA editing sequence (MEGA- PRESS) was used to accurately assess GABA levels in this volume. MRS data were processed and quantified as linear combinations of the spectral patterns of each brain metabolite using LCModel software. We found that 1) as a group, the Mn-ex- posed subjects have GABA levels significantly elevated by 55% (p<0.01) in the basal ganglia volume; 2) the concentration of NAA in the frontal cortex is decreased in exposed subjects compared to controls (p=0.04), and within this exposed group decreases with increasing cumulative Mn exposure (R=−0.93, p=0.01); 3) Glutamate+Glutamine/Cr in the basal ganglia of exposed subjects decreases with increasing PI (R=−0.648, p<0.05). Using logistic regression, combining the PI with the GABA level yields a biomarker to predict exposed versus non-exposed subjects with 98% accuracy. (Supported by DoD USAMRMC W81XWH-05-10239).

**1001 ENDOPASMIC RETICULAR STRESS INDUCED BY SINGLE METHYLMERCURY EXPOSURE IN RAT BRAINS: TIME COURSE AND REGION SPECIFICITY.**

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Methylmercury (MeHg) is a global environmental hazard and its neurotoxic ac- tions continue to be of concern. Though MeHg triggers free radical formation and induces oxidative stress, the exact mechanisms of its toxicity remain unclear. This study was designed to explore the time-course and region-specific alterations in en- doplasmic reticulum stress induced by single exposure to MeHg in Sprague-Dawley rat brains. Forty-two rats were randomly assigned to 7 groups, and were injected ip with 0 or 4 mg MeHg/kg body weight. Thirty min, 1 hr, 3 hr, 6 hr, 12 hr or 24 hr after injection, animals were decapitated and cortex, cerebellum, hippocampus, striatum and brain stem were dissected out. Expression of Grp78, a marker of endoplasmic reticulum stress, was determined by western blot analysis. Among brain regions of interests, the time-course of expression of Grp78 was analogous. Grp78 expression peaked at 6h after injection and then decreased and returned to control level at 24 hr after injection. However, when compared to normal controls,
increased Grp78 expression was statistically significant vs. controls in the cortex and brain stem of MeHg treated animals (p<0.05). Specifically, the expression of Grp78 in the cortex was significantly higher at the earliest time point (1 h) after injection. With regard to the magnitude of Grp78 increase, the induction in cortex was the most pronounced among the five studied regions, and the peak at 6 h after injection was increased by more than 150% of normal control levels. These studies suggest that endoplasmic reticulum stress is involved in MeHg-induced neurotoxicity and that the effect is region specific.

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With regard to the magnitude of Grp78 increase, the induction in cortex was the most pronounced among the five studied regions, and the peak at 6 h after injection. With regard to the magnitude of Grp78 increase, the induction in cortex was the most pronounced among the five studied regions, and the peak at 6 h after injection was increased by more than 150% of normal control levels. These studies suggest that endoplasmic reticulum stress is involved in MeHg-induced neurotoxicity and that the effect is region specific.

While manganese (Mn) is required for many biological functions and enzyme activities, excessive chronic exposure to Mn leads to a neurological disorder, called Manganism. Although the mechanism of Mn-induced neurotoxicity has not been completely established, recent evidence suggests that glial cells play a crucial role in mediating its neurotoxicity as it induces impairment of glutamate transporters in astrocytes. Several studies suggest that TGF-β (E2) is neuroprotective in animal and cell models. Tamosifen (TX) is also neuroprotective in various experimental settings. However, the mechanisms involved in E2- and TX-induced neuroprotection have yet to be elucidated. We hypothesized that E2 and TX protect Mn-induced neurotoxicity by enhancing glutamate transporter function. Two glutamate transporters, GLAST and GLUT-1, play a central role in preventing excitatory neurotoxicity by removing excess glutamate from the synapse into astrocytes. Herein, we studied the ability of E2 and TX to enhance GLUT-1 expression or function via the transforming growth factor-β (TGF-β) pathway. Primary astrocytes from rat cortical regions were used. The results show that Mn decreased GLUT-1 expression at both the mRNA and protein levels, while E2 and TX increased its expression and attenuated the Mn-induced reduction of GLUT-1. E2 at 5, 10 and 20 nM increased GLUT-1 protein expression by 26.4, 48.7 and 49.5%, respectively. TX at 1 and 2 μM increased GLUT-1 by 42.6 and 57%, respectively. Mn decreased TGF-β expression, whereas E2 and TX increased its expression in astrocytes. TGF-β enhanced [3H]glutamate uptake by 51.5% in astrocytes and increased GLUT-1 expression. These results suggest that the protective effects of E2 and TX on Mn-induced GLUT-1 impairment are mediated, at least in part, by enhancement of TGF-β pathway signaling in astrocytes.

The World Health Organization estimates that 65–80% of the world’s population use traditional medicine as their primary form of health care. As the incidence in disease states affecting women increases, a corresponding increase in the use of complementary and alternative medicines (CAM) has been observed. In the Asian cultures, CAM has had a long history of development and application in the treatment of many diseases affecting multiple organ systems. Approximately 38% of adults in the U.S. currently use some form of CAM therapy (20% of women in the U.S. use some form of CAM therapy for control of menopausal symptoms or other related health concerns alone), some of which are used in conjunction with conventional medicine. In 21st century medicine, the value of CAM has been considered and questioned due in part to the use of advanced technologies in bringing novel insights into the unique features of CAM. However, there are safety concerns in the use of CAM that may interfere with conventional medicine or pose unique safety risks for susceptibility to other disease states. Presenters in this session will discuss the use of CAM to improve women’s health. Specific topics that will be addressed are the impact of CAM on breast and endometrial cancers and menopause in addition to the advantages and disadvantages of CAM in each health related paradigm. The overall goal of this session is to highlight the current status of toxicology issues in the use of CAM in women’s health. In addressing these issues, we are hopeful that attendees will develop a better appreciation of the use of CAM and the challenges that arise with their use with respect to safety.

Scientists do science, writers write. Wrong! Scientists do science and write about it as well. It is imperative that scientists publish their work. Furthermore, publishing is just one aspect of science. Scientists also have to be able to communicate complex scientific concepts to the non-scientific audience. This large group of constituents include the general public, media, policymakers, communities, and individuals. This is an obligation scientists have towards the community-at-large and one that can be accomplished with relative ease once the basic nuances of effective communication are understood. Effective communication is therefore, not just an icing on the cake; rather it is fundamental to interpretation and dissemination of science. Yet scientists are not natural communicators and many have no formal training in science writing. They learn to write by following the style and approach of their mentors or other authors. Some form of training in science writing becomes even more crucial for authors for whom English is a second language. Laying this basic foundation is important since the public learns about science from many different sources, including newspapers, magazines, books, radio, television, the Internet, electronic news services, and films. Because information is readily available at our finger tips it can easily be distorted with the unfortunate circumstance that bad science sometimes triumphs over good science. Therefore it is important for us to effectively communicate science messages to distinguish the myths from the facts. This session will aim to highlight strategies, techniques, and resources that make the field of good science communication invaluable.

In human (FIH) doses. As an extension to last year’s biotherapeutics roundtable discussion on the concepts of when a Minimal Anticipated Biological Effect Level (MABEL), a Pharmacologically Active Dose (PAD), or No Observable Adverse Effect Level (NOAEL) approach should be considered for setting FIH doses, this session will discuss application and clinical validation of the various approaches. Selection of the most appropriate approach is dependent on a clear understanding of the target biology and pharmacology of the biopharmaceutical in question in pharmacologically relevant animal species and/or animal models of disease and appropriate human in vitro systems that can better predict the outcome in humans. In cases where immune activation is desired, exaggerated immune responses could lead to adverse immune related events (e.g. cytokine release, systemic inflammatory response, and organ failure) that in some cases may have serious consequences. Similarly in cases where immune modulation is desired to combat autoimmune inflammatory disease the exaggerated pharmacology can result in immunosuppression resulting in serious infections that in some cases have also been fatal. Immune antagonist targets may also be considered high risk in causing unintended immune activation and may warrant MABEL or alternate preclinical strategies for estimating FIH dosing. A retrospective analysis of estimated FIH doses for both immune agonist and antagonist classes of immunomodulator biopharmaceuticals in clinical development will be presented and compared to the eventual dose used in the clinical trial to demonstrate safety and efficacy. Case examples of when MABEL vs. NOAEL vs. PAD approach was relevant to FIH dosing will be discussed.
1007 RISK ASSESSMENT OF EXTRACTABLES FROM PLATINUM-CURED SILICONE TUBING MATERIALS USED IN THE MANUFACTURING OF BIOLOGICS.


Evaluation of extractables and leachables is of increasing importance because of their potential impact on product quality attributes which relate to the safety and efficacy of biologics. The extractables of 3 medical grade platinum-cured silicone tubing materials, used in production transfer systems, were investigated: one in current production use and two as possible alternates. All materials conformed to USP Class VI, ISO 10993 and PhEU compendial requirements: Extraction studies were conducted in model solvents (50°C for 4 days). Organic and inorganic compounds extracted were analyzed by HPLC-UV, LC-MS, ICP-MS and GC-FID-MS techniques. The results showed predominantly linear polydimethylsiloxane (PDMS) structures and elemental silicon (Si) common to all three materials. The PDMS extraction processes, i.e., solvation molecular descriptors and a linear solvation energy relationship was obtained; log(k) = -2.41 - 0.18 R + 1.76 P + 0.018 A - 3.38 B + 6.91 V, n = 28, R2=0.94, F =72. From the re-leasing rates of the candidates PRCs, it was known that compounds having log(k) values of the PRCs were considered measurable releases in a one month period of passive sampling, which could be used as PRCs for sorbent based PSDs after deuterated labeling.

1008 SELECTION OF PERFORMANCE REFERENCE COMPOUNDS VIA SOLVATION ENERGY DESCRIPTORS FOR SORBENT BASED PASSIVE SAMPLING DEVICES FOR POLAR ORGANIC POLLUTANTS IN WATER.

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Performance reference compounds (PRCs) are critical references in passive sampling of aquatic pollutants to correct the effects of natural turbulence, temperature and bioaffecting. However, there is no proper PRC available for sorbent based passive sampling devices (PSDs) designed for newly emerged polar organic pollutants from pesticides, herbicides, pharmaceuticals and household chemicals. The existing PRCs based on hydrophobicity for passive sampling of hydrophobic compounds cannot be used for the sorbent PSDs because of the strong retaining strengths of the PRCs to have measurable releases. We hypothesized that proper PRCs for the sorbent based PSDs can be selected based on the molecular interactions forces between the passive sampling processes, i.e., lone-pair electrons (R), polarity/polarizability (P), hydrogen-bond donor (A), hydrogen-bond receptor (B) and hydrophobicity (V). The adsorption coefficients (k) of 30 PRC candidates were measured and the re-leasing rates of the PRCs were measured in a flow system. The log(k) values of the PRCs were scaled to their known solvation molecular descriptors and a linear solvation energy relationship was obtained; log(k) = -2.41 - 0.18 R + 1.76 P + 0.018 A - 3.38 B + 6.91 V, n = 28, R2=0.94, F =72. From the re-leasing rates of the candidates PRCs, it was known that compounds having log(k) values of the PRCs were considered measurable releases in a one month period of passive sampling, which could be used as PRCs for sorbent based PSDs after deuterated labeling.

1009 CHEMICAL RISK ASSESSMENT OF PICRAMIC ACID — A SURROGATE APPROACH.

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Traditional human health risk assessment relies primarily on in vivo bioassays to provide information for hazard identification and dose-response assessment. However, environmental contaminants and industrial chemicals often have limited data suitable for characterization of toxicity and associated dose-responses for cancer and non-cancer effects. Picramic acid is a prime example of such a chemical in that it has no in vivo repeated dose toxicity data for conducting a conventional risk assessment. Picramic acid, a breakdown product of the explosive chemical ammonium picrate and often present at munitions sites, is of significant interest to U.S. EPA’s Superfund Program. In the present investigation, we demonstrate the utility of an in vitro approach for picramic acid risk assessment by prioritizing, monitoring, and testing of environmental chemicals.

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High-throughput, lower-cost, in vitro toxicity testing is currently being evaluated for use in prioritization and eventually for predicting in vivo toxicity. Interpreting in vitro data in the context of in vivo human relevance remains a formidable challenge. A key question in using in vitro data to predict in vivo toxicity is whether dosimetry is sufficient to establish dose-response relationships. In this study, hepatic toxicity data in the context of in vivo human relevance remains a formidable challenge. A key question in using in vitro data to predict in vivo toxicity is whether dosimetry is sufficient to establish dose-response relationships. In this study, hepatic toxicity data in the context of in vivo human relevance remains a formidable challenge. A key question in using in vitro data to predict in vivo toxicity is whether dosimetry is sufficient to establish dose-response relationships.
bioactivity based on predicted oral equivalents and estimated human exposures could be interpreted as a higher priority for further testing and monitoring. Approved for publication but does not necessarily reflect Agency policy.

1011 GENOTOXICITY TESTS CONDUCTED ON A GROUP OF STRUCTURALLY RELATED ALDEHYDES. S. Bhatia, V. T. Politianna and A. Apin. RIFM, Woodcliff Lake, NJ.

To assess genotoxicity potential, Ames assays and/or in vivo mouse micronucleus tests (MMNT) using NMRI mice were conducted on a group of 6 structurally related aldehydes (Hexen-2-al; 2-Nonenal; 2-Dodecanal; (E)-4-Decenal; 10-Undecenal; Benzaldehyde) that are used as fragrance materials. All the studies were conducted according to OECD Testing Guidelines and GLP. The Ames assay (pre-incubation and plate incorporation method) was conducted using Salmonella typhimurium strains TA98, TA100, TA102, TA1535, TA1537 in the presence and absence of S9. 2-Dodecanal, (E)-4-Decenal and 10-Undecenal (high doses ranging from 1000 to 5000 μg/plate in the presence and absence of S9) were negative in the Ames test. Hexen-2-al was positive only in one strain TA100 in the absence of S9 in the pre-incubation assay but negative in the presence and absence of S9 in the plate incorporation assay. Benzaldehyde and (E)-4-Decenal induced micronuclei in all the six aldehydes that were tested (Hexen-2-al; 2-Nonenal; 2-Dodecanal; (E)-4-Decenal; 10-Undecenal; Benzaldehyde) no significant increases in micronuclei were observed. It is concluded that these aldehydes are not genotoxic.

1012 CALCULATIONS FOR HYPER-ACTUE, HIGH-CONCENTRATION INHALATION EXPOSURES. S. N. Chesler1,2, J. Moser2 and H. Salem2. Chemical Security Analysis Center, Department of Homeland Security, Aberdeen Proving Ground, MD, 2Battelle Memorial Institute, Columbus, OH and 3Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD.

Effectively calculating exposure dose following high concentrations of inhalable noxious gases requires tools, not ordinarily used in acute toxicology. These methods include: 1) utilization of toxic load concepts when calculating total exposure dose, and 2) allometric scaling to apply animal-derived toxicological data to human populations. At extremely high concentrations, Haber’s Law likely does not apply due to unique mechanisms that occur at these concentrations. Examples of these mechanisms include lachrymation, deliquescing chemical burns, and ocular and high doses ranging from 1000 to 2000 mg/kg. Approximately 24 and 48-hr after dosing, the bone marrow cells were collected for micronucleus analysis. At least 2000 poly-chromatric erythrocytes (PCEs) per animal were scored for micronuclei. In all the six aldehydes that were tested (Hexen-2-al; 2-Nonenal; 2-Dodecanal; (E)-4-Decenal; 10-Undecenal; Benzaldehyde) no significant increases in micronuclei were observed. It is concluded that these aldehydes are not genotoxic.

1014 PROVISIONAL ADVISORY LEVELS (PALS) FOR TEAR GAS (CS).

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PALS values developed by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures for hazardous materials corresponding to three severity levels and durations of 24 hrs, 30 and 90 d, and 2 yr durations. PAL 1, 2, and 3 severity levels represent the threshold for mild effects, serious/irreversible/escape-imparing effects, and lethal effects, respectively. PALS have not been promulgated nor have they been formally issued as regulatory guidance; they are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. The PAL protocol has been applied to estimate oral and inhalation exposure limits for Tear Gas (CS). CS is a potent irritant, with symptoms of exposure including lacrimation, blepharospasm, erythema of the eyelids, chest tightness, coughing, nasal irritation and discharge, salivation, throat irritation, nausea, vomiting, and cutaneous irritation. It is reported that an aerosol concentration of 4 mg/m3 will disperse the majority of rioters within 1 minute, and 10 mg/m3 will deter trained troops. With the exception of more severe cutaneous reactions, recovery from exposure is usually rapid upon exposure to fresh air, generally within 30 minutes after exposure. Data were available for deriving oral PAL 2 and 3 values for 24 h, and inhalation PAL 1, 2, and 3 values for 24 h, 30, 90 d, and 2 yr. Data were insufficient for derivation of an oral PAL 1 value for 24 hours, and of oral PAL 1, 2, and 3 values for 30 d, 90 d, and 2yr. PALS estimates to be presented were based on evaluation of experimental data in humans and animals, and were approved by the Expert Consultation Panel for Provisional Advisory Levels in October 2008.

1015 ACUTE STUDIES OF INHALED CHLORINE IN F344 RATS SUGGEST ALTERNATIVE TO HABER’S RULE FOR RISK EXTRAPOLATIONS.

T. S. Peay1, J. McKee1, G. A. Willson2, M. H. George1, R. H. Jaskot1, D. G. Ross1, T. M. Moore1 and A. M. Jarabek1. 1U.S. EPA, Research Triangle Park, NC, and 2EPL, Inc., Research Triangle Park, NC.

Chlorine (Cl2), a high-production volume air toxic, is an irritant of interest to homeland security. Risk assessment approaches to estimate egress or re-entry levels use an assumption based on Haber’s Rule and apply a concentration times duration (“C x t”) adjustment to extrapolate across exposure scenarios. We conducted a set of acute exposure studies to explore this assumption. Male and female rats were exposed whole-body to inhaled Cl2 for 1 hour at 0, 6, 30 and 60 ppm; 6 hours at 0, 1.0, 1.5, 5.0 and 10 ppm; and 24 hours at 0.25, 1.25, and 2.5 ppm. “C x t” equivalent levels (ppm x hr) are 0, 0.6, 6, 30 and 60 ppm. Endpoints indicative of epithelial disruption were evaluated, including tissue histopathology immediately post-exposure and biochemistry, such as cellular evaluation of lavage fluids at 24-h post-exposure. Noses were sectioned transversely to provide six standard section levels. Biochemical analyses of nasal lavage (NAL) and bronchoalveolar lavage (BAL) fluids included lactate dehydrogenase, N-acetyl-β-D-glucosaminidase, and total protein (TP). The expected proximal to distal distribution of lesions (inflammation, necrosis, degeneration and hyperplasia) with increasing concentration levels was observed but the type, incidence and severity of each did not follow a “C x t” pattern. Duration of exposure was a dominant determinant for the emergence of both inflammation and hyperplasia. Inflammation tended to follow necrosis or degeneration. Both concentration and duration were determinants of olfactory degeneration. The dose-re-
and H. Cle

G. Ross1, T. M. Moore1 and T. S. Peay1.

spectively. The “C x t” equivalent levels were 0, 0.6, 6.0, and 15 ppm-hr. Noses studies were 0, 1.0 and 10 ppm; 0.1, 1.0 and 2.5 ppm; and 0, 0.1 and 1.0 ppm, re-
exposure study after the 5-d exposure coincided with the necropsy of the 10-d (6
necropsy of the 5-d (6 hr/d, 5 d/w) exposure, and the recovery period of the stop-
period for the stop-exposure study after the 6-hr exposure coincided with the
(George et al., SOT 2010) studies of inhaled chlorine (Cl2) in female F344 rats was
stract does not reflect Agency policy).

Chlorine (Cl2) is very reactive in water and a respiratory tract (RT) irritant. Lesions in the RT show a proximal to distal distribution determined by concentration, but roles for airflow, mucus flow and tissue susceptibility are indicated. Our hypothesis is that irritant effects of Cl2 are due to oxidative stress mediated by hypochlorous acid (HOCl). HOCl is formed in tissues by hydrolysis of Cl2, and by downstream responses such as inflammation. To better understand the pathogenesis of inhaled Cl2 and provide an important link between new acute mechanistic studies (Peay et al., SOT 2010) and the extant 2-yr bioassay (Wolff et al., 1995), we performed a 5- and 10-d study. Female F344 rats were exposed whole-body 6 hr/d, 5 d/w to inhaled Cl2 for 5 d at 0, 0.1, 0.5, 1.0 and 2.5 ppm; or 10 d to 0, 0.1, 0.5, and 1.0 ppm. These concentrations coincide with those of the 2-yr bioassay (1.0 and 2.5 ppm) and extend the exposure levels below the lowest in the 2-yr bioassay (0.4 ppm) as no no-observed-adverse-effect level (NOAEL) was identified. “C x t” equivalent expo-
ure levels are 0, 0.6, 3, 6 and 15 ppm-hr. Tissue histopathology was performed im-
mediately post exposure and biochemical or cellular evaluation of lavage fluids at 24-hr post exposure. Noses were sectioned transversely to provide six standard sec-
tion levels. Biochemical analyses of nasal lavage and bronchoalveolar lavage fluids included lactate dehydrogenase, N-acetyl-β-D-glucosaminidase, and total protein. Inflammation and hyperplasia, not observed in the acute studies, dominated the observed lesions in both studies. Also unlike the acute studies, there was no in-
volvement of the olfactory epithelium. Goblet cell metaplasia emerged at 10 d in a concentration dependent manner. Hyperplasia of the nasopharyngeal duct only oc-
curred at the 15 ppm-hr level of the 5-d study. These findings suggest that refined approaches to duration adjustment for risk assessment of different exposure scenar-
ios are necessary to map the dynamic pathogenesis of inhaled irritants. (This ab-
tract does not reflect Agency policy).

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As part of a program to inform approaches for risk assessment of inhaled irritants of interest to homeland security, a set of acute (Peay et al., SOT 2010) and subacute (George et al., SOT 2010) studies of inhaled chlorine (Cl2) in female F344 rats was performed. The design included equivalent exposure levels when calculated based on a daily “C x t” product (ppm-hr). Additionally, stop-exposure studies were included that allowed for comparison across different study durations: the recovery period for the stop-exposure study after the 6-hr exposure coincided with the necropsy of the 5-d (6 hr/d, 5 d/w) exposure, and the recovery period of the stop-

This work has been supported by the National Institute of Environmental Health Sciences, Research Triangle Park, NC. As part of a program to inform approaches for risk assessment of inhaled irritants of interest to homeland security, a set of acute (Peay et al., SOT 2010) and subacute (George et al., SOT 2010) studies of inhaled chlorine (Cl2) in female F344 rats was performed. The design included equivalent exposure levels when calculated based on a daily “C x t” product (ppm-hr). Additionally, stop-exposure studies were included that allowed for comparison across different study durations: the recovery period for the stop-exposure study after the 6-hr exposure coincided with the necropsy of the 5-d (6 hr/d, 5 d/w) exposure, and the recovery period of the stop-

This work has been supported by the National Institute of Environmental Health Sciences, Research Triangle Park, NC.
Office of Environmental Health Hazard Assessment (OEHHA) is developing reference exposure levels (RELs) for MDI and TDI to protect against the adverse effects of repeated and chronic exposures. Because of the severity of response among sensitized persons, choosing RELs that prevent sensitization in the first place is a prudent approach to health protection but may not protect sensitized individuals. While RELs for 8-h exposures are usually higher than chronic RELs, formaldehyde is an example of a chemical whose sensitizing potential dictated a lower 8-hr REL. Long term exposures of 50 and 500 ppb caused allergic sensitization (Thraher et al., 1990). For formaldehyde, the chronic REL of 9 μg/m3 (7 ppb) is based on an occupational study in which irritation caused nasal obstruction and lower airway discomfort, but with no evidence of sensitization. To protect against sensitization with repeated exposures, the 8-hr REL was also set at 7 ppb. Similar considerations apply to MDI and TDI. Based on a NOAEL of 0.9 ppb and a LOAEL of 1.9 ppb for decreases in lung function (FEV1) in TDI workers, we derive a chronic REL of 0.004 ppb (0.026 μg/m3). This includes a subchronic uncertainty factor (UF) of 3, a toxicokinetic UF of 3.14, and a toxicodynamic UF of 10. In workers with isocyanate-induced asthma, challenge at a level of 1 ppb for a total of 47 ppb/min isocyanates induced asthmatic responses in 3 of 8 subjects (Lemiire et al., 2002). This value is 10% of the sensitization concentration. Comparing this with a REL of 0.004 ppb suggests that this REL will protect against initial sensitization and a subsequent hypersensitive response.

1021 EXPOSURE AND HEALTH RISK ASSESSMENT FOR CHILDREN AND ADULTS POTENTIALLY EXPOSED TO BROMINATED FLAME RETARDANTS ON TELEVISIONS AND IN HOUSE DUST.
F. Shay1, A. Burn1 and E. Sweet2, ChemRisk, LLC, Pittsburgh, PA and 2University of Michigan SPH, Ann Arbor, MI.

The significance and potential sources of chemicals detected in house dust and their relative contribution to total human uptake of environmental contaminants have become topics of recent interest within the field of risk assessment. Brominated flame retardants (BFRs), which are used in a variety of consumer products in the home, have been receiving increased scientific scrutiny and public interest surrounding their detection in the indoor environment and in humans. There is particular concern with respect to children’s exposures in the home because children spend the majority of their time indoors and have more frequent mouthing activities. In this evaluation, an exposure assessment and quantitative risk assessment was performed for BFRs that have been associated with house dust due to their use in television (TV) components. Specifically, this assessment focuses on a recent study that measured BFRs in Japanese TV sets. Using U.S. EPA guidelines we estimated carcinogenic risks and non-carcinogenic hazards for toddler and adult residents to Decabromodiphenyl ether (DecaBDE), Tribromobiphenyl A (TBBPA), and Hexabromocyclododecanec (HBCD). Results for toddlers indicate that direct contact exposures to BFRs in TV dust could result in a non-cancer hazard index of 0.038 (for all three BFRs evaluated) and a theoretical increased cancer risk of 7 x 10^-9 (DecaBDE only). For comparison purposes, a separate risk assessment was also conducted for DecaBDE detected in house dust using data from published literature. While the Japan TV and general house dust estimates are not directly comparable, the results of this evaluation suggest that residential exposure to BFRs from TVs would not present unacceptable health risks to adults or children.

1022 THE AFFECTS OF TEMPERATURE AND HUMIDITY ON DIACETYL MEASUREMENTS.

Inhaled diacetyl, a component of butter flavorings, has been shown to be responsible for adverse health effects in microwave popcorn workers and animals. Sampling devices and methodologies for quantitative exposure levels of diacetyl have been shown to be a function of temperature and humidity in the sampling environment. The objective of this investigation was to develop a vapor calibration system (VCS) to calibrate sampling devices under a wide variety of environmental conditions. A custom flow-temperature-humidity controller allowed accurate control of the diluent air input into the VCS. The liquid of interest was injected into a heated port where it was vaporized. The mixed vapor and air were then passed into a Teslon bag. The temperature around the bag was regulated to ensure that the temperature and humidity inside the bag were maintained at user-defined levels and to prevent condensation on the inner walls of the bag. After equilibration, sampling instruments were utilized to precisely control environmental conditions. Custom data acquisition and control software was developed to automate the calibration process. The real-time response of a set (n=4) of volatile organic meter photo-ionization detectors (MINIRAE 2000) were calibrated for diacetyl with the VCS. Diacetyl concentrations of 5, 75 and 150 PPM were examined at temperatures of 66, 78 and 90°F and relative humidities of 5, 30 and 50%. Results indicated a correction factor of 0.75 + 0.12*exp(0.11*AH) needed to convert BFRs detected by the VCS to the correct diacetyl concentration (AH = absolute humidity mg/L). Future uses for the VCS include calibrating other sensors and sampling methodologies along with different vapors.

1023 METHYL FORMATE AS A SUBSTITUTE BLOWING AGENT FOR PLASTICS.
L. E. Collins, A. G. Salmon and M. A. Marty. OEHHA, CalEPA, Oakland, CA.

Concerns about smog formation, ozone depletion, and global warming have prompted the desire to reduce use of, or to replace, specific, currently used chemicals. Often very little is known about the toxicity of the substitute chemical. Because of the smog forming properties of alkanes and alkenes, methyl formate (CAS 107-31-3) has been proposed as a partial substitute for n-butan, isobutene, and isopentane in their use as blowing agents in plastics manufacturing in California. If allowed by regulators, increased exposure to workers and to the general public near facilities using methyl formate will occur. Methyl formate has been in use for more than 70 years and is not expected to be less irritating to mucous membranes than its metabolites, formaldehyde and formic acid. In the body methyl formate is hydrolyzed to methanol and formic acid. Methanol is oxidized to formaldehyde and then to formic acid. At high levels internal toxicity could occur. At doses likely to be achieved in environmental exposure, toxicity appears to get minor. OEHHA has derived an interim acute (1-hour) Reference Exposure Level (REL) of 0.93 ppm for methyl formate based on effects on the human nervous system. Although derived by approved methodology, the REL for methyl formate has not undergone external peer review.

1024 PROVISIONAL ADVISORY LEVEL (PAL) DEVELOPMENT FOR MALATHION.

PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-imparing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for malathion to estimate oral and inhalation exposure limits, as experimental data permit. Malathion is a broad-spectrum organophosphorous insecticide with a wide variety of uses. Pharmacokinetics of malathion are influenced by the degree of carbonyl mercury hydrolysis in mammalian tissues. The acute neurotoxic action of malathion is cholinergic. Estimated lethal oral doses to humans range from 350-2000 mg/kg. Subchronic inhalation exposure to a concentration that caused eye and nose irritation did not result in ChE activity inhibition. Inhalation exposure of laboratory animals caused plasma and RBC ChE activity inhibition in the absence of clinical signs. A reliable lethality study was not found for inhalation exposure. The LD50 values in rats ranged from 1000-1400 mg/kg and in mice were 1430-3500 mg/kg for different strains. In longer term oral studies with rats or mice, ChE activity inhibition was measured in the absence of clinical signs; at high doses, body weight gain was decreased, gastric lesions were observed, and mortality in male rats was increased. PAL estimates, based on evaluation of experimental data in humans and rats, were approved by the Expert Consultation Panel for Provisional Advisory Levels in April 2008 and will be presented.

1025 EFFECTS OF DECOSAHAEANOIC ACID ON DEVELOPMENTAL METHYLMERCURY TOXICITY IN MICE: NEUROBEHAVIOURAL IMPACTS.

Methylmercury (MeHg) persists as an environmental neurotoxicant with adverse effects particularly noted in the developing brain. The main source of MeHg into the human food chain is via seafood consumption. However, fish is also an impor-
tant source of n-3 fatty acids such as docosahexaenoic acid (DHA) which has neuoprotective effects, and which plays an important role during the prenatal devel-
opment of the central nervous system. The aim of the present study was to study the effects of DHA on development MeHg toxicity using behavioural endpoints in a mammalian model. A battery of neurobehavioural analyses were performed on 15d-old mice which had been exposed to varying levels of DHA and MeHg throughout development via the maternal diet. There were six exposure groups: Control; MeHg (as its naturally-occurring cysteinate form; ~ 4 mg/kg); low DHA (~ 9.5 mg/kg); high DHA (~ 29 mg/kg); MeHg + low DHA and MeHg + high DHA. Supplementation of the maternal diet with DHA reduced MeHg accumu-
lalion in brains of mice offspring. This effect was unrelated to the level of DHA supplementation. DHA accelerated the development of grasping reflex in a dose-
dependent manner in mice offspring. Effects were also noted on grip strength, a measure of both physical and behavioral development. Pups from dams fed with ‘MeHg + low DHA’ showed lower grip strength. This effect seemed to disappear in pups from dams fed with ‘low DHA’. A significant interaction between ‘MeHg’ and ‘low DHA’ was also observed. Development of physical markers and some of the early pup behavioral parameters (righting, rooting, clasp and auditory startle) did not seem to be impacted by the exposure groups. The results from the present study show the potential of DHA in alleviating toxicity caused by MeHg, contributing towards refining risk/benefit assessments and towards a better understanding of neurodevelopmental discrepancies found between epidemiological studies.

1026 IN UTERO AND LACTATIONAL EXPOSURE TO 2, 3, 7,
8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)
INDUCED ALTERATIONS IN HUMAN PROSTATE GLANDS
AND FIBROSIS IN RHESUS MONKEYS.
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We investigated the effects of TCDD exposure on the prostate in rhesus monkey
offspring. Dams received 0, 30 or 300 ng/kg TCDD subcutaneously on Day 20 of
gestation, and then 5% of the initial dose was injected every 30 days until Day 90
after delivery. The offspring were maintained until reaching sexual maturity, and
examined histopathologically. Dose-dependent decreases in the prostate glands
and widespread fibrosis were observed in offspring. It is noteworthy that 7 years from
the final lactational TCDD exposure, inflammatory cell infiltration and disruption of
the glandular epithelium were also observed, and indicated these changes were considered
not to be regressive. Furthermore, we conducted global gene expression
analysis by microarray analysis. As a result, the number of genes with a greater than
1.5-fold change and statistically significant differences in mRNA expression was
1502. Among these differentially expressed genes, we classified 5 categories
(Fibrogenesis, Inflammatory response, Disruption of cell component, and
confirmed quantitatively with real-time quantitative
expression patterns were simi-
lar between microarray analysis and real-time quantitative PCR. These results indi-
cated that fibrosis in the prostate were ongoing changes in offspring and had caused
prostatic dysfunction. This prostatic dysfunction caused by TCDD exposure is con-
cidered associated with the findings in our previous reports, i.e., reductions in sperm
and semen quality in second-generation rhesus monkeys.

1027 EVALUATION OF ORGAN WEIGHT DATA FOR
RODENT TOXICITY STUDIES.
S. Jana, M. A. Mullu, S. K. Pandey, A. Govindarajan, V. Goyal, S. Ingle and R.
Nirgi. Toxicology, Saven Life Sciences Limited, Hyderabad, Andhra Pradesh, India.
Sponsored by Y. Fdw, 2SRC Inc., North Syracuse, NY.

Comparison of organ weights between control and treatment groups is indeed an
important quantitative endpoint in toxicity studies and has conventionally been
used to evaluate the toxic effect of the test article. A statistical analysis is performed
to estimate a treatment effect on the organ weight. The objective of this work is to
understand the relationship between organ weight, body weight and brain weight
as well as to identify parameter which best predict a true effect of chemical on organ
weights. Materials for the present evaluation are comprised of control animal data
collected from short-term repeated dose oral toxicity studies conducted in Wistar
and Sprague-Dawley rats at Saven Life Sciences. All the organ weight data were
subjected to linear regression and correlation was established with body weight
and brain weight. Degree of correlation was determined on the basis of ratio between
correlation coefficient (r) and probable error (PE). If the ratio (r/PE) was more than
6, correlation was considered significant. Significant correlation between body
weights and weights of liver, kidneys and heart was noticed in the present data set.
Correlation with brain weight was also evident for these organs but it was more in
Wistar than Sprague-Dawley rats. Similarly, spleen weights were also correlated
with body weights only in Wistar rats. Thyroid-parathyroid weights were more cor-
related with brain weights than body weights. Sex biasness in weights of adrenals
was observed where females only revealed a significant correlation with body
weights in either strain. Other organs did not show any correlation neither with
body weights nor with brain weights. In conclusion, liver, kidneys, heart, spleen
and adrenals weights relative to body weight could be considered for toxicity pre-
diction whereas in case of thyroid-parathyroid weights, relation to brain weight
should be used. For organs like testes/ovaries, pituitary gland and brain, either ab-
solute weight or other alternative statistical methods should be identified.

1028 PROVISIONAL ADVISORY LEVEL (PAL)
DEVELOPMENT FOR FENAMIPHOS.
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Corporation, North Syracuse, NY and 4U.S. EPA, Washington, DC.

PAL values developed for hazardous materials by the U.S. EPA represent general
public emergency exposure limits for oral and inhalation exposures corresponding
to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-
year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the
threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the
threshold for lethal effects. PALs have not been promulgated nor have they been
formally issued as regulatory guidance. They are intended to be used at the discre-
sion of risk managers in emergency situations when site-specific risk assessments
are not available. Application of PAL protocols has been performed for fenamiphos to
the degree supported by the available data. Fenamiphos is an organophosphate
ne-maticide and insecticide, for which all registrations in the United States were can-
celled on or before May 31, 2007. Fenamiphos inhibits cholinesterase activity in humans and other mammals, and, as a result, it can overstimulate the nervous sys-
tem to cause nausea, dizziness, confusion, and, at very high exposures, respiratory
paralysis and death. It is readily absorbed from the gastrointestinal tract and is also
absorbed through inhalation and through intact skin. Oral PAL values were derived
from feeding studies on dogs and rats and from gavage studies on rats. Data were
available to support derivation of oral PAL values at all durations for PAL 1,
through 90 days for PAL 2, and only for 24 hours for PAL 3. Inhalation PAL values were
derived from inhalation studies on rats that involved either a single 4-hour ex-
posure or repeated 6-hour exposures spread over three weeks. These data supported
derivations of all three PAL values for 24 hours and a PAL 1 for 30 days. PAL esti-
mates for fenamiphos were approved by the Expert Consultation Panel for
Provisional Advisory Levels in July 2008 and will be presented.

1029 ATSDR’S ACUTE- AND INTERMEDIATE-DURATION
ORAL MINIMAL RISK LEVELS (MRLs) FOR
ACRYLAMIDE.
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2SRC Inc., North Syracuse, NY.

In 2008, about 141,000 metric tons of acrylamide were produced in the USA.
Human exposed to acrylamide via oral route mainly ATSDR has derived acute- and
intermediate-duration oral MRLs of 0.02 mg/kg/day and 0.002 mg/kg/day, respec-
tively, for acrylamide during the development of a new Toxicological Profile for
Acrylamide. The MRLs are based on results of animal studies because adequate
data for human data are not presently available. Various human variability were applied. Results of available animal studies identify male-
directed reproductive effects and neurological effects in orally-exposed rats as the
most sensitive nonneoplastic effects of acute-duration oral exposure to acrylamide.
These effects were elicited in Long-Evans rats following oral dosing of males
for 5 days and subsequent matings with untreated females. Benchmark dose (BD) values were set at their 99% confidence limits (99% CL) using a bench-
mark response of 10% to determine a point of departure for deriving the MRL.
Default uncertainty factors for extrapolation from animals to humans and for
human variability were applied. Results of available animal studies identify male-
directed reproductive effects and neurological effects in orally-exposed rats as the
most sensitive nonneoplastic effects of acute-duration oral exposure to acryl-
amide. Ultrastructural degenerative peripheral nerve changes have been detected
at lower exposure levels than those eliciting male-mediated reproductive effects.
Therefore, the intermediate-duration oral MRL for acrylamide is based on degener-
avite nerve changes in orally-exposed rats as the critical effect. A NOAEL/LOAEL

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approach was selected because results of the ultrastructural evaluations included only 3 of 10 rats/group and were reported only as the total numbers of fields (per group) with ultrastructural changes as axolemma invaginations or Schwann cell without axons and/or with degenerating myelin. These study limitations precluded BMD analysis. Default uncertainty factors for extrapolation from animals to humans and for human variability were applied to the identified NOAEL for degenerative nerve changes.

**1030 ASSESSING HUMAN HEALTH RISK FROM EXPOSURE TO N, N-DIPHENYL-1, 4-BENZENEDIAMINE.**


DPPD is an antioxidant/stabilizer for rubber, oils, foodstuff, and a component of rubber mixtures used for fabrication of dental prostheses. One case study (Conde-Salazar et al., 2004) of repeated occupational exposure to a mixture containing DPPD, N-cyclohexyl-N′-phenyl-4-phenylenediamine and N-isopropyl-N′-phenyl-4-phenylenediamine was associated with hypertensive lesions. The toxicological database for DPPD includes a poorly reported rodent chronic toxicity study which identifies a LOAEL of 194 mg/kg-day based on reduced serum calcium levels and increased renal calcification in male rats (Hasegawa et al., 1989). Available reproductive studies in mice and rats examined few toxicological endpoints and are limited by incomplete reporting. Several studies used vitamin E-deficient diets, confounding the reproductive toxicity findings of DPPD exposure. Despite their limitations, these studies provide consistent evidence that DPPD exposure prior to and through gestation results in frank effects (maternal mortality and still births) at doses as low as 2.5 mg/kg-day. Maternal mortality was possibly associated with larger fetuses causing parturition difficulty and mortality. A reproductive NOAEL of 0.3 mg/kg-day (Draper et al., 1956, 1958) was identified in rats. However, the frank effects occurred at a dose only about 8-fold higher than the NOAEL. This steep dose response causes concern for hazard assessment. Quantitative Structure-Activity Relationship analysis (TOPKAT® software) predicted a rat LOAEL (273.5 mg/kg-day) beyond model’s optimum prediction space (OPS), and thus was unreliable. Using this and other examples, the presentation will further examine similar steep dose response relationships for reproductive/developmental endpoints and their impact on hazard assessment. (The report does not constitute views and policies of the U.S. EPA).

**1031 CYTOXICITY OF MIDDLE EASTERN DUST IN RAT LUNG EPITHELIAL CELLS.**

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U.S. troops deployed to the Middle East are often exposed to dust storms with particulate matter (PM) levels above the military exposure guideline of 150 µg/m³. Our goal is to characterize the toxicity of the soluble metal components of Middle Eastern PM. Replicating rat type II alveolar epithelial cell cultures (RLE-6TN) were maintained in DME media, with 10% fetal bovine serum and 1% antibiotic-antimycotic solution and grown at 37°C with 5% CO₂. Exposures of confluent cell monolayers of the liver (cholangiocarcinoma, hepatocholangioma and hepatocellular carcinoma) evidence of carcinogenic activity of PCB118 based on increased incidences of neo-plasms of the liver (cholangiocarcinoma, hepatocholangioma and hepatocellular adenoma) and cystic keratinizing epithelioma (CKE) of the lung. Based on data from this and a recent study of TCDD and other DLCs conducted by NTP under similar conditions, we used sigmoidal dose-response modeling to calculate relative potency factors for PCB118, for endpoints seen in common, under conditions of common shape of the respective dose-response curves. The relative potency of PCB118 for induction of cholangiocarcinoma, hepatocellular adenoma, and CKE was, 0.00004, 0.0001 and 0.00003 respectively. These data are consistent with the current WHO(2005) TEF of 0.00003 for PCB118, used in cancer risk assessments.

**1032 TOXICITY AND HEALTH HAZARD ASSESSMENT FOR SYNTETIC PARAFFINIC KEROSENE.**

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The U.S. Air Force is pursuing the development of alternative fuels to decrease dependency on foreign oil sources. One jet fuel, designated as Synthetic Paraffinic Kerosene (SPK), is produced from natural gas using the Fischer-Tropsch (FT) process. While 98% of SPK components are found in JP-8, SPK contains a higher percentage of isoparaffins, and no aromatic compounds such as BTX and naphthalene. The toxicology research program included a dermal irritation study, two in vitro genotoxicity tests and an in vivo genotoxicity test. Inhalation studies conducted included acute, two-week ringer/toller, 90-day toxicity and sensory irritation RD50. Dermal irritation was slight to moderate and all genotoxicity studies were negative. The acute inhalation study with both sexes of rats at 2000 mg/m³ for 4 hours resulted in no abnormal clinical observations. Male and female F344 rats were exposed for 6 hours per day, 5 days per week, for 90 days to an aerosol-vapor mixture of SPK set fuel (0, 200, 700 or 2000 mg/m³). Effects on the nasal cavities were minimal (700 mg/m³) to mild (2000 mg/m³) while only the high dose produced multifocal inflammatory cell infiltration in rat lungs (both sexes). The RD50 in mice was 10,900 mg/m³. Based on the results of the toxicity studies combined with the comparative health hazard assessment (HHA), SPK appeared moderately less toxic or irritant than JP-8. An Occupational Exposure Limit (OEL) of 200 mg/m³ was proposed, the same OEL as JP-8. The toxicity research program. HHA and OEL for SPK are expected to form the baseline for comparing future synthetic and biomass-derived alternative fuels under development by the Air Force. Supported by AFMC 77 AESW/LF.

**1033 CARCINOGENIC POTENCY OF 2, 3', 4, 4', 5-PENTACHLOROBIPHENYL (PCB118) IN FEMALE HARLAN SPRAUGE-DAWLEY RATS.**

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The dioxin Toxic Equivalency Factor (TEF) approach is currently used worldwide for assessing and managing the cancer risks posed by exposure to mixtures of polychlorinated dibenzodioxinoids (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). While the TEF approach is applied to both non-ortho and mono-ortho substituted PCBs there have been no long-term cancer bioassays of any individual mono-ortho substituted PCBs. To address this knowledge gap, the National Toxicology Program conducted a series of 2-year rodent bioassays of dioxin-like compounds (DLCs) in female Harlan Sprague Dawley rats, including a study of 2,3,4,5-tetrachlorobiphenyl (PCB118) conducted under conditions of the respective dose-response curves. The relative potency of PCB118 was >99% and that the non-PCB118 TEF contribution of synthetic byproducts was 0.39ngTEQ/mg PCB118 bulk material. Following chronic exposure to PCB118 (5 days/wk) by gavage at doses up to 4.6 mg/kg there was clear evidence of carcinogenic activity of PCB118 based on increased incidences of neo-plasms of the liver (cholangiocarcinoma, hepatocellular carcinoma and hepatocellular adenoma) and cystic keratinizing epithelioma (CKE) of the lung. Based on data from this and a recent study of TCDD and other DLCs conducted by NTP under similar conditions, we used sigmoidal dose-response modeling to calculate relative potency factors for PCB118, for endpoints seen in common, under conditions of common shape of the respective dose-response curves. The relative potency of PCB118 for induction of cholangiocarcinoma, hepatocellular adenoma, and CKE was, 0.00004, 0.0001 and 0.00003 respectively. These data are consistent with the current WHO(2005) TEF of 0.00003 for PCB118, used in cancer risk assessments.

**1034 PROVISIONAL ADVISORY LEVELS (PALS) DEVELOPMENT FOR OXAMYL.**


PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for...
and P

2 years with an adequate number of survivors. Administration for mortality or in-life data for both sexes. Additionally, as the terminal mortality against time (1998-2009) did not reveal any significant trends in mortality values for dietary and oral gavage studies were similar, with the mortality is dependent on the form of the administered chemical: the dust being more toxic than the aerosol. For purposes of risk assessment, chronic exposure to oxamyl is considered a series of daily acute exposures. Oral and inhalation PAL estimates, based on evaluation of experimental data in humans and rats, were approved by the Expert Consultation Panel for Provisional Advisory Levels in July 2009, and will be presented.


Reviews of Sprague-Dawley, Cr:CD® BR (VAF) International Generic Standard (IGS), rat tumorigenicity studies completed between 1998 and 2000 indicated that the strain showed a high mortality pattern in comparison with other rat strains, particularly in females. The purpose of this review of up to 33 studies, completed up to 2009, was to establish if there were any changes in the mortality, bodyweight and food consumption data over time or between the dietary and oral gavage routes of administration. The animals were mainly gang housed and low protein diet was used in all the studies. The results have shown that the mortality values at 2 years are still high (mean ±8.5%, males and 67±5.7%, females). Analyses of the terminal mortality against time (1998-2009) did not reveal any significant trends in females, but there was an indication of a slight increase in the males. The terminal mortality values for dietary and oral gavage studies were similar, with the mortality over 2 years also showing a similar pattern for both routes of administration. There were no major differences over time or between the routes of administration for bodyweight growth pattern/gain and food consumption data. In conclusion, there is an indication that the terminal mortality for males has slightly increased over time, although formal statistical significance was not attained, but there was no indication of any major differences over time for females or between the routes of administration for mortality or in-life data for both sexes. Additionally, as the terminal mortality remains high (particularly in females) it is advisable to employ a strategy, such as increasing group size, to satisfy regulatory requirements of reaching 2 years with an adequate number of survivors.


Quantitative assessment of behavioral patterns is used in rodent toxicity studies. For monkeys, only limited approaches are available such as qualitative behavioral scoring (CBS) using care sensors (2) or video analysis (3). Behavioral studies using human observations have low inter-observer reliability and poor reproducibility. This work investigates if quantitative behavioral monitoring used for rodents can be applied to macaques. Video-tracking EthoVision® XT software (Noldus) and special analysis software (Delta Phenomics) were used to evaluate amphetamine (1 mg/kg, 2 mL/kg) and diazepam (0.3 mg/kg, 1 mL/kg) related behavioral changes in pair/group housed animals (n=6). Recordings were made for 30 min predose and at anticipated Tmax. Analysis of multiple parameters comprised distance moved, velocity and mobility. Amphetamine increased the distance moved (males [m]: +61%, females [f]: +63%), velocity (m: +117%, f: +113%) and mobility (m: +62%, f: +23%) compared to baseline. Amphetamine enhanced the duration of the highly mobile phase and baseline duration of immobility of females was 2- to 3-fold lower then in males. Diazepam provoked only minimal behavioral changes, probably due to low dose used and confounding effects of previous treatment. The automated individual recognition followed by semi automated analysis is more reliable and efficient than subjective human observation. Furthermore, monkeys can be observed in their enriched home cage. The extensive set of movement features and the specific combination of some of those features, even allows the quantification of tremor-like movements. The results of this feasibility study will accelerate implementation of the described technology to be used in general toxicology and (safety) pharmacology studies aimed at the detection and comparison of clinically relevant or adverse treatment effects. 1. Korte S et al. J Pharmacol Toxicol Mds 56: 47 (2007); 2. White A et al. Neurobiol Aging 27 (10): 1477 (2006) 3. Spruijt BM et al. Drug Disc Today: Technologies 3: 251 (2006)

1037 COMPARATIVE SAFETY OF RECENTLY REGISTERED NEW ACTIVE INGREDIENTS IN CALIFORNIA. C. N. Aldous and P. Leung. Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.

Department of Pesticide Regulation (DPR) in Cal-EPA reviews toxicity studies on pesticidal active ingredients (AI’s). Of 46 “new” AI’s registered at Cal-EPA since 2007, 14 had recently completed full data sets of mammalian toxicity studies (such as chronic, reproductive, and developmental toxicity). All other “new” AI’s were excluded from this analysis because they were either designated by U.S. EPA as “reduced risk” chemicals or ubiquitous and of presumed low toxicity, or were not novel chemicals (such as renewals of lapsed registrations). The 14 new AI’s with full data sets contained a few studies flagged by DPR reviewers as “possible adverse effects.” Rat tumor increases in lifetime studies were observed with orthosulfamuron (thyroid follicular cell adenomas in males), metolfluthrin (hepatocellular tumors in both sexes), spirodifen (testicular interstitial cell adenomas and uterine adenocarcinomas), and sulfosulfuron (2 bladder transitional cell tumors in females only: rare and considered treatment-related). Mouse oncogenicity studies indicated elevated lung tumors in both sexes with folicamid and fluopicolide, urinary bladder tumors in males, and with sulfosulfuron, and hepatocellular tumors in both sexes with tenoxon. All above oncogenicity studies in both species found elevated tumors associated with high dose levels and/or predisposing non-neoplastic lesions. The reproduction study for spirodifen found male infertility, pup body weight decrements, and post-implantation losses at a high dose level that was toxic to adults. An unacceptable reproduction study for tribenuron-methyl was flagged for adverse effects, which were not reproduced in a FITRA guideline study. Metofluthrin dog chronic and rat subchronic neurotoxicity studies were flagged as “adverse,” but only due to sustained acute effects such as tremors. Data indicate acceptable risks for pesticides registered since 2007.

1038 PAHS AND DIOXINS NOT PRESENT IN FLY ASH AT LEVELS OF CONCERN. L. J. Bradley, E. Perry, K. Vosnakis and C. Archer. AECOM, Westford, MA.

Coal ash is the material remaining after coal burns, and fly ash is the fraction that exits the combustion chamber in the flue of the boiler and is collected in air pollution control systems prior to atmospheric release. Although numerous published reports have demonstrated that polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzodioxins/dibenzofurans (dioxins/furans) are not present in fly ash at appreciable levels, some regulators continue to require a site-specific demonstration. The results from ten fly ash samples analyzed for PAHs and dioxins/furans are presented and compared to conservative risk-based human health and ecological screening levels. The comparison indicates that all PAH concentrations in all 10 samples were below the conservative screening levels. The dioxin/furan data were evaluated on a toxic equivalency (TEQ) basis using U.S. EPA-approved toxic equivalency factors (TEF). All 10 results were below the human health TEQ screening level, and the majority of the samples (8 of 10) were below mammalian- and avian-based ecological TEQ screening levels. The findings suggest that expensive analyses for PAHs and dioxins/furans for future fly ash site investigations may be unnecessary and unwarranted.


Manufactured gas plants (MGP) were important sources of gas for light and heat from the 1850s through the 1950s. MGP plants also generated waste that contaminates soil and groundwater, and there is an ongoing need to evaluate potential environmental and human health risks at former MGP sites. In addition to polycyclic aromatic hydrocarbons and benzene/toluene/ethylbenzene/xylene, cyanide (CN) is frequently identified as a contaminant of concern at former MGP sites. Although
free CN is highly toxic, most CN at former MGP sites is found in ferrocyanide (Fe-CN) compounds. These compounds, which can give soil a bluish appearance, are much less toxic than free CN, and release free CN only under certain chemical and environmental conditions. Hence, an understanding of model chemistry and site environmental conditions is important for evaluating potential human health risks of CN at former MGP sites. We conducted a screening-level assessment of human health risks at a hypothetical former MGP site, using upper bound estimates of media concentrations and intake parameters. Thus our analysis is based on parameters likely to overestimate actual risks. We estimated chronic exposure to free CN in soil, groundwater and air, and to Fe-CN in soil and groundwater. We also evaluated acute exposure to free CN in soil, for a pica child. We used published toxicity criteria to evaluate potential risks for free-CN. For Fe-CN, we derived toxicity criteria based on a sub-chronic drinking water study in rats. For chronic exposure, we estimated a total hazard index of 0.8, with ingestion of Fe-CN in ground U.S. contributing approximately 50%, and ingestion and inhalation of free-CN each contributing approximately 25% to the total hazard. Potential acute exposure to free CN in soil for a pica child is also below the published toxicity criterion. Although concentrations of total CN can be relatively high at former MGP sites, our screening level analysis indicates that the CN and chemistry of CN, as well as environmental conditions, are such that CN is not likely to represent a human health hazard at most former MGP sites.

1040 RISK ASSESSMENT OF VALERIAN ROOT EXTRACT AS A FOOD INGREDIENT.
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Valerian root, and extracts thereof, has been consumed since antiquity as a food flavoring agent and for a variety of suggested therapeutic activities. However, a “full book” of safety studies recommended to determine the safety of food ingredients, as outlined in the FDA Redbook, have not been completed for valerian root extracts. Therefore, a risk assessment was necessary to determine safety-in-use as a flavoring agent, and needed to be comprehensive in nature, taking into account both the preclinical studies conducted on the food ingredient and its constituents, as well as the history of use. Valerian root extract is obtained from the dried roots or rhizomes of the Valeriana officinalis L. plant. Substances found in the valerian root extract include iridoid valepotriates (e.g., valtrates and valerosidate), volatile essential oil constituents (e.g., bornyl isovalerenate, valeric acid, and monoterpenes and sesquiterpenes), alkaloids and lignans. In toto, the plant contains over 150 chemical constituents, many of which are physiologically active. The valerian root has a long history of use as a sedative and in the treatment of headaches and high blood pressure. Currently, the primary use of valerian root extract as a food flavoring ingredient for addition to alcoholic and nonalcoholic beverages, baked goods, breakfast cereals, hard and soft candies, condiments, gelatin puddings, and frozen dairy products. Per capita consumption estimates find that valerian root extract is consumed as a food ingredient at 0.0016 mg/kg body weight/day, while consumption from supplements may be near 17 mg/kg body weight/day. Acute and short-term studies conducted on valerian root extract, and carnosinogenicity and reproductive and teratogenic studies in animals and its individual constituents indicate a low level of toxicity. Several of the valtrates contained in valerian root extract are not mutagenic, although in vitro studies indicate cytotoxicity at low levels. Based on studies on valerian root extract and its individual constituents, as well as the long history of human consumption, valerian root extract can be considered safe when consumed as a food ingredient at current use levels.

1041 DERIVATION OF CHEMICAL-SPECIFIC AND GENERIC BLOOD-BASED BIOMARKER SCREENING CRITERIA FOR VOLATILE ORGANIC COMPOUNDS (VOCs) - APPLICATION OF STEADY-STATE PBPK MODEL SOLUTIONS.
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The National Health and Nutrition Examination Survey (NHANES) has measured volatile organic compounds (VOCs) in blood collected in the general U.S. population. However, interpretation of blood-based biomonitoring data for VOCs is hampered by the lack of health risk-based screening criteria expressed in terms of blood concentration. We tabulated current exposure guidance values such as U.S. Environmental Protection Agency (EPA) reference doses and concentrations (RfDs, RfCs) and collected key chemical-specific PBPK model parameters for approximately 50 VOCs that are amenable to headspace analysis in blood samples. We used published steady-state solutions to the generic PBPK model to estimate blood concentrations of parent compound predicted in humans under continuous exposure at the available RfD or RfC values. Based on these calculations, we exam-ined the distribution of predicted steady-state blood concentrations associated with existing health-based screening criteria across VOC compounds. The blood concentrations were highly correlated with the exposure guidance values across a wide range (over three orders of magnitude). We propose values for screening criteria for evaluation of blood-based biomonitoring data on a chemical-specific basis for approximately 50 VOCs. Based on the distribution of these values and available physical/chemical property data, we propose a tiered decision-tree approach and generic blood-based screening criteria for additional VOCs, similar in concept to the Thresholds of Toxicological Concern (TTC) approach used to evaluate intake of trace chemical residues in foods. Such screening values can enable biomonitoring data for VOCs to be used in a risk assessment and risk management framework to assist in prioritization for research and risk assessment follow-up.

1043 A 26-WEEK REPEAT-DOSE TOXICITY STUDY IN CYNOMOL-GUS MONKEYS WITH XOMA 052, A NOVEL MONOCONAL ANTIBODY TARGETING IL-1 BETA.
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XOMA 052 is an ultra-high affinity (300 fM) humanized IgG2 monoclonal antibody that specifically binds to IL-1 beta (IL-1β) and inhibits activation of the IL-1 receptor. This activity is expected to prevent the cellular signaling events that produce inflammation. IL-1β is a proinflammatory cytokine involved in the development of many diseases including Type 2 diabetes, rheumatoid arthritis, gout. XOMA 052 has been evaluated in two Phase 1 clinical studies in Type 2 diabetes designed to assess safety, pharmacokinetics and measures of glycemic control and systemic inflammation. It was well tolerated and demonstrated clinically meaningful biologic activity in diabetes and systemic inflammatory assessments. The cynomolgus monkey was selected for safety evaluation due to similar binding affinity and in vitro functional activity between human and monkey IL-1β with XOMA 052. XOMA 052 was administered by subcutaneous injection weekly for 27 weeks at doses up to 90 mg/kg, with a recovery period of 3 months. The nonclinical safety evaluation included daily clinical observations, food consumption, body weight, hematology, clinical chemistry, coagulation parameters, flow cytometry, urinalysis, ophthalmic examinations, electrocardiography (EKG), heart rate/blood pressure, T cell-dependent antibody response (T DAR), toxicokinetics, immunogenicity, as well as macroscopic and microscopic pathology. The results showed no biologically significant findings and the no-observed-adverse-effect level (NOAEL) was considered to be 90 mg/kg. This study supports the phase 2 clinical evaluation of XOMA 052 in patients with Type 2 diabetes as well as other chronic indications.

1044 NONCLINICAL TOXICOLOGY EVALUATION OF A MONOCONAL ANTIBODY AGAINST THE VEGF CO-RECEPTOR NEUROPILIN-1.
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Neuropilin-1 (NRP1) is a multifunctional receptor that contributes to development of the nervous and vascular systems. NRP1 was first described as a receptor that binds semaphorin 3A, acting with the plexins to regulate neuronal guidance. It was later shown that NRP1 also binds vascular endothelial growth factor (VEGF) to mediate vascular development. Anti-NRP1 is a phase-derived human IgG1 monoclonal antibody (mAb) against human NRP1 that blocks its binding to VEGF, but not to semaphorins, and therefore reduces in vivo angiogenesis and vascular remodeling without interfering with neuronal guidance. Anti-NRP1 has an additive effect with anti-VEGF to reduce tumor growth in mouse xenograft models and is being evaluated as a potential therapy for cancer. Unlike many mAbs, anti-NRP1 binds to NRP1 from numerous species, including mouse, rat, cynomolgus monkey, and human. Since the rat is an accepted rodent species for use in IV toxicity studies and anti-NRP1 demonstrated comparable binding affinity across species, rats and cynomolgus monkeys were chosen to study the safety of anti-NRP1. A pilot toxicology study was conducted in rats; anti-NRP1 was administered IV once every 3 days for 15 days (total of 5 doses) at dose levels of 0, 10, or 50 mg/kg. GLP toxicology studies were conducted in rats and cynomolgus monkeys; in both species, anti-NRP1 was administered IV once weekly for 8 weeks (total of 9 doses) at dose levels of 0, 10, 30, or 100 mg/kg. Anti-NRP1 was well tolerated. Significantly lower serum triglycerides were observed following anti-NRP1 administration in both species. No gross or microscopic correlates were noted, and this effect was not considered toxicologically significant. The results of these studies indicated that the no observed adverse effect level was ≥ 100 mg/kg. These studies enabled entry into human clinical trials and demonstrated the feasibility and utility of the two species approach to testing the nonclinical safety of this mAb.
Vitamin D and its analogs demonstrate a range of biological activities that suggest possible efficacy in cancer chemoprevention and therapy. QW1624F2-2 (1-hydroxyethyl-16-ene-24,24-difluoro-25-hydroxy-26,27-bis-homovitamin D3) is a bifunctional vitamin D analog that both (a) activates signaling pathways downstream of the vitamin D receptor, and (b) inhibits CYP24 (the enzyme responsible for vitamin D catabolism). In a 28-day toxicity study, groups of 4 beagle dogs/six received daily oral (gavage) exposure to Q at doses of 0, 1, 5, or 10 μg/kg/day. Due to body weight loss in the high dose group, the 10 μg/kg/day dose was reduced to 2.5 μg/kg/day during week 2. No mortality was seen in any dose group. Dogs exposed to Q at ≥ 2.5 μg/kg/day demonstrated increased kidney weights and decreased thymus weights. Histopathologic evaluations identified mineralization in the stomach and kidneys, and C-cell hyperplasia in the thyroid as treatment-related lesions in dogs receiving Q at ≥ 2.5 μg/kg/day; mineralization was interpreted as secondary to hypercalcemia in these animals. Exposure to Q at ≤ 1 μg/kg/day was not hypercalcemic, and had no effects on body weight, clinical signs, food consumption, clinical chemistry parameters, hematology parameters, organ weights, or gross pathology. The only possibly agent-related toxicity of Q at 1 μg/kg/day was minimal mineralization in the stomach and kidney; this dose was considered to be the No Observed Adverse Effect Level (NOAEL) for subchronic oral administration of Q in dogs. (NCI-N01-CN-43040).
SAFETY EVALUATION OF MM-111, A NOVEL BISPICIFIC MOLECULE TARGETING ERBB2 AND ERBB3.

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MM-111 is a novel, bispecific scFv antibody fusion protein designed to inhibit tumor growth that was derived from computational modeling of the epidermal growth factor (EGF) pathway. MM-111 consists of human anti-ErbB2 scFv and anti-ErbB3 scFv antibodies joined by a modified human serum albumin (HSA) linker. These safety studies support a Phase I/II clinical study of administering single escalating doses of MM-111 to patients with advanced, refractory cancers. MM-111 was administered at dose levels up to the maximum feasible dosages (based on the clinical dose formulation of 25 mg/ml), following repeat-dose (once or twice weekly for up to four weeks) 1 hour intravenous infusions administered to Sprague Dawley (SD) rats or cynomolgus monkeys with a 2-4-week treatment-free recovery period. The SD rat and cynomolgus monkey have been shown to have target receptor homology, MM-111 binding activity and tissue cross-reactivity. Toxicity was assessed by evaluating mortality, morbidity, clinical observations, body weights, food consumption, physical and ophthalmic examination observations, ECGs, clinical pathology, urinalysis, local tolerance, organ weights, and macroscopic and microscopic anatomic pathology. The only MM-111 related finding in some animals administered 30 and 250 mg/kg MM-111 (5 doses over 4 weeks) was a minimal to moderate periductular mixed cell infiltrate in various tissues, primarily gastrointestinal and reproductive, which did not impair function. There were no other MM-111 related effects. The periductular and periairular findings were largely reversible after a 4-week treatment free recovery period in rats and monkeys, respectively. These studies established a NOAEL of 250 mg/kg in rats and 30 mg/kg in monkeys.

BENCHMARKING THE BILE SALT EXPORT PUMP (BSEP), VESICLE TRANSPORT ASSAY.

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The bile salt export pump (BSEP) is an efflux transporter, driving the elimination of substrates from hepatocytes into the bile, and is primarily responsible for the elimination of monovalent, conjugated bile salts (such as taurochenodeoxycholate, taurocholate, glycocholate, and others). Disruption of BSEP activity through genetic disorders is known to manifest in clinical liver injury such as progressive familial intrahepatic cholestasis type 2. Drug-induced disruption of BSEP is hypothesized to play a role in the development of liver injury for several marketed or withdrawn therapeutics, including bosentan, erythromycin estolate, CI-1034, CP-724,714, AMG 009, and others. Standard preclinical animal models, such as rodents, have been poor predictors of the liver injury associated with BSEP interference in humans. In vitro screening systems exist to quantitatively assess a compound’s ability to interfere with BSEP function. In the absence of a relevant preclinical in vivo model for BSEP-mediated liver injury, applicability of in vitro models to human toxicity require the evaluation of benchmark compounds with known clinical outcomes. In this study, membrane vesicles harvested from BSEP-transfected insect cells were used to assess the activity of more than 200 benchmark compounds. Test articles were assessed at 10 concentrations per compound, with IC50 values derived from non-linear regression analysis. Based on this data, it appears possible to associate BSEP potencies (IC50 values) to liver liabilities in humans. Although the most accurate translation of risk would incorporate pharmacological potency, PK and physico-chemical properties, indication, and other drug attributes, the additional understanding of a compound’s potency for BSEP interference should help to limit or avoid BSEP-related liver liabilities in humans – which are typically not detected with standard preclinical animal models.

A COMPREHENSIVE SUMMARY OF THE EFFECTS OF OSELTAMIVIR IN JUVENILE RABBITS.

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The antiviral oseltamivir is currently approved for the treatment and prevention of influenza in children older than 1 year of age. In order to support its development in infants aged less than 1 year, several non-clinical studies were undertaken. Acute oral administration of oseltamivir phosphate (OP) was well tolerated by adult rodents and 14-day old juvenile rats at 2000 mg/kg (based on phosphate salt). No adverse effects were observed in 7-day old juvenile rats at 500 mg/kg/day dosed for 14 days in two subsequent studies, while doses starting from 700 mg/kg/day were not tolerated by all animals after the first dose. More recently, another single oral gavage study in 7-day old rats was conducted including a functional observational battery. No test item related findings were observed at 394 mg/kg. Effects at higher doses (≥ 657 mg/kg) were considered to be indicators of general toxicity. The lack of specific CNS effects was in line with the finding that the brain exposures for oseltamivir and its active metabolite oseltamivir carboxylate (OC) were relatively low, with brain/plasma ratios always well below one. Based on the correlation between tolerability and plasma exposure, oseltamivir, but not OC, appeared to underlie the general toxicity observed. This was confirmed in a separate study in 7-day old rats using subcutaneous dose administration of 25 and 50 mg/kg (based on free base) of OC alone, which resulted in the highest maximum plasma and brain OC levels thus far observed in 7-day old rats, but did not induce any effects. Based on the current data in juvenile rats and plasma exposure measured in young infants (6-8 months), the safety margins for oseltamivir and OC are greater than 100-fold. In conclusion, oseltamivir was well tolerated in juvenile rats down to 14 days old, in 7-day old juvenile rats general toxicity occurred after oral administration of very high supratherapeutic doses only resulting in large safety margins, therefore supporting the clinical development in infants aged less than 1 year.

GENE EXPRESSION PATTERN FROM RATS TREATED WITH ATYPICAL ANTIPLATFORMOTIC DRUGS AS A MECHANISM-BASED SIGNATURE FOR PREDICTING DRUG-INDUCED INSULIN RESISTANCE.


The objective of this study was to investigate the role of changes in expression of genes in the insulin receptor signaling pathway on the physiologic endpoint of acute insulin resistance induced by treatment of rats with certain atypical antipsychotic drugs. In order to determine the impact of acute exposure to these drugs on insulin resistance, we performed an in vivo glucose tolerance test (IVGTT) on male Sprague Dawley rats following a single SC injection of the second generation (atypical) antipsychotic drugs risperidone, olanzapine, or aripiprazole. At the tested doses, olanzapine and aripiprazole, but not risperidone, caused an inhibition of insulin-stimulated glucose uptake. In a parallel study, rats were administered a single SC dose of the atypical antipsychotic drugs at the same doses used in the IVGTT. Animals were sacrificed thirty minutes after dosing and gastrocnemius muscle was collected for RNA extraction and gene expression analysis. Eighty-four genes annotated to the insulin receptor signaling pathway were profiled by real-time RT-PCR in order to characterize gene expression changes correlating with drug-induced insulin resistance. In addition to investigating the mechanisms underlying the observed insulin resistance, we identified candidate biomarker genes to be used as a predictive gene signature. Gene expression analysis identified a list of fifteen genes that showed inverse expression patterns between the positive- and negative-class compounds. Of the fifteen genes comprising the signature, thirteen genes were repressed by the positive class compounds while only two were induced. Genes repressed by olanzapine and aripiprazole but not risperidone include fatty acid synthase, leptin, P13-Kinase, PPAR-gamma, PKC, and resistin. These findings suggest that atypical antipsychotic drugs which induce acute insulin resistance correlate with repression of genes in the insulin receptor signaling pathway and this gene expression pattern may be useful as a predictive biomarker.

COMPARATIVE STUDY ASSESSING DIFFERENT PROCEDURES FOR INTRAMUSCULAR INJECTION IN THE RABBIT.

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Safety assessment studies with vaccines administered via the intramuscular (im) route often involve the use of the rabbit, since a full human dose can be injected in that species. In this study, various injection procedures and subsequent retrieval of the injection sites for microscopic examination were evaluated. Groups of three males each were injected im with Al(OH)3 in the calf muscle (depth of 5 or 13 mm), in the paravertebral muscle, or in the anterior thigh muscle. The groups of males each were injected im with Al(OH)3 in the calf muscle (depth of 5 or 13 mm), in the paravertebral muscle, or in the anterior thigh muscle.
sites collected 3 days after injection were used to determine the most appropriate procedure for imm injection of rabbits. No treatment-related changes were observed in clinical signs or body weight. The inoculation procedures and retrieval of the injec-
tion sites at necropy were performed without major difficulties. The injection of the calf muscle at a depth of 5 mm resulted in the delivery of a lower dose-vol-
ume because of reflux. For the anterior thigh and calf muscles, palpation was con-
sidered to be the most convenient way of determining the injection site. The com-
bination of palpation and measuring the distance from the spinal cord was best for the paravertebral muscle. No abnormalities were observed macroscopically at the the injection sites. As expected, microscopic examination revealed that Al(OH)₃ induced a mononuclear type inflammation consisting predominantly of medium-
sized macrophages and monocytes, while inoculation with saline induced a slight inflammation of mononuclear cell type, predominantly small-sized macrophages. The retrieval of lesions induced by Al(OH)₃ was most successful in the anterior thigh muscle, followed by the calf muscle, but was distinctly less successful with the paravertebral muscle. In conclusion, the anterior thigh muscle was considered to be the best im injection site for the nonclinical toxicological evaluation of vaccines in rabbits.

**1055** QUALIFICATION OF CARDIOPULMONARY SAFETY PHARMACOLOGY MONITORING METHOD USING TELEMETRY IN CYNOMOLGUS MONKEYS EVALUATED WITH PHARMACOLOGICAL MODULATORS.

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**Sponsor:** G. Wacher

Combined cardiovascular and respiratory monitoring are routinely performed in separate studies but there are strong scientific incentives to obtain the measurements from both systems in the same animals. Six female cynomolgus monkeys were surgically implanted with telemetry transmitters recording ECG (DII), arte-
tial pressure, physical activity, body temperature, respiratory rate and tidal volume. Respiratory rate (RR) and tidal volume (TV) were monitored simultaneously with the telemetry system and with a heated pneumotachometer connected to a helmet with bias flow. Control drugs with cardiovascular and respiratory effects, including saline, medetomidine, cocaine and remifentanil, were administered intravenously. Medetomidine and remifentanil induced significant cardiovascular and respiratory depression with decrease in RR and TV. As expected, cocaine increased TV and minute ventilation (MV) with concomitant increase in heart rate. The onset, dura-
tion and magnitude of cardiovascular and respiratory pharmacodynamic changes were significantly correlated. In addition, interpretation of results highlighted the dependency of both the cardiovascular and respiratory systems. The use of car-
diopulmonary monitoring can potentially increase sensitivity of safety pharmacol-
ogy investigations by allowing interpretation of test article effects on both systems. Correlation between respiratory monitoring by telemetry and by spirometry was in-
vestigated for a range of RR and TV. This model of cardiovascular and respira-
tory monitoring in conscious cynomolgus monkeys could be useful to fulfill regula-
ry requirements for safety pharmacology testing as defined in the ICH S7A guideline.

**1057** PRECLINICAL SAFETY EVALUATION OF CMX157: A LIPID-CONJUGATED NUCLEOTIDE ANALOG FOR TREATMENT OF HIV.


Though widely used, tenofovir disoproxil fumarate (TDF) has reduced activity against several common HIV mutants and a risk of nephrotoxicity. CMX157 is a hexadecylxyporphyrin lipid conjugate of tenofovir (TFV) with activity against wild-
type and drug-resistant HIV. Unlike TDF, CMX157 is not efficiently cleaved to free TFV in the blood. This should increase the levels of active TFV-diphosphate in target cells and reduce the rate of secretion in the kidney. To support human ad-
ministration, studies were conducted in rats and dogs to identify undesired phar-
macological effects on the cardiovascular, CNS, respiratory, renal and gastrointesti-
nal systems. Mitochondrial toxicity was evaluated in HepG2 cells using the Complex IV/Flouraxin ratio. Finally, in vitro and in vivo genotoxicity and 28-day rat and monkey studies were completed. A single dose of 1000 mg/kg resulted in de-
creased body temperature and urine volume noted for the presence of occult blood. Effects on respiration rate, urine chemistry and hemodynamic parameters were ob-
erved at 200 and 1000 mg/kg. Due to the small magnitude and/or transient nature of these changes, they were not considered biologically important. In both rats and monkeys, clear effect and NOAEL doses were identified with good exposure after oral administration. Gastric effects resulted in mortality due to dehydration at doses of 600 and 800 mg/kg/day in rats and monkeys, respectively. In monkeys, these effects reversed during a 7-day dose holiday after which dosing resumed suc-
cessfully at 400 mg/kg/day. Observations included excess salivation, emesis and di-
arhea. Slight elevations in ALT and BUN occurred in individual animals at doses above 200 mg/kg/day. Anatomic pathology changes were minimal and generally limited to the stomach. The NOAEL was 200 mg/kg/day in both rats and mon-
keys. The gastric effects occurred at doses that greatly exceed those planned for ini-
tial human studies. No important safety pharmacology effects and no mitochondri-
al or genotoxicity was observed. Therefore, preclinical testing supports the clinical development of CMX157.

**1056** FUNCTIONAL AND ANATOMIC CONSEQUENCES OF SUBRETINAL DOSING IN THE CYNOMOLGUS MACAQUE.

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There is growing interest in subretinal delivery of therapeutic agents for treatment of ocular disease. In this study, we characterized functional and anatomic conse-
quences of the subretinal injection per se using multifocal electroretinography (mERG), optical coherence tomography (OCT), immunohistochemistry (IHC) and transmission electron microscopy (TEM). The right eyes of 3 cynomolgus macaques were given subretinal injections (100 μl) of a balanced salt solution (BSS) in the supertemporal macula. The control (left) eyes received intravitreous injec-
tions (100 μl) of BSS. Fundus photography, OCT and mERG were obtained be-
fore and immediately after injection and again 9 and approximately 21, 35, 63 and 90 days later. IHC for glial fibrillary acidic protein (GFAP), rhodopsin, S-cone

opsin and M/L-cone opsin, and TEM of retina were also done. Results at all post-
 injection intervals were similar to baseline in eyes given intravitreous injections. Expected mild retinal hemorrhage occurred at the subretinal injection sites. Transient suppression of mERG responses occurred in the bleb and also in other non-bleb regions. Retinas reattached by 2 days after injection. Partial recovery of mERG amplitudes occurred by 9 days post-injection and was largely, but not com-
pletely recovered after 90 days. OCT showed a marked decrease in the inner seg-
ment/outer segment (IS/OS) line. There was no change in GFAP and S-cone stain-
ing but rhodopsin and M/L-cone opsin were partially displaced into the inner segments. TEM revealed disorganization of the rod outer segment (but not cone) discs. Subretinal injections were well tolerated although some residual effects re-
mained after 90 days. Transient suppression of mERG responses spatially remote from the bleb, was unexpected. While mERG responses nearly recovered, disor-
ganization of the rod outer segment discs remained and may explain the decrement in the IS/OS line on OCT seen in the region of the former blebs.

**1058** TOXICITY STUDIES OF COCHLOSPERMUM TINCTORIUM AQUEOUS ROOT EXTRACT IN WISTAR RATS.

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**Sponsor:** B. Gadashibu.

Inspite of advances in the understanding of the medicinal properties of many herbs, the consumer today is confronted with lack of or misinformation concerning the safety of these herbs. In the present study the toxicity of the aqueous root extract of Cochlospermum tinctorium was investigated because of its use in the treatment of diarrhea. The administration of the aqueous root extract at the limit dose of 3000 mg/kg body weight orally to Wistar rats, (acute study) using up-and-down method, did not produce death in the treated animals. The prolonged administration of the
extract to four groups of five rats each at 50, 100, 200 and 400 mg/kg body weight doses for 21 days produced decrease in red blood cell count and haemoglobin concentration. This also increased the value of packed cell volume, which may indicate megaloblastic anaemia. The observed increase in the white blood cell counts probably indicated possible stimulation of the immune system. There was significant (p<0.05) increase in the values of creatinine and urea suggesting the extract may be toxic to the kidney. The histopathology study however, revealed some changes in the liver, kidney, spleen, lungs, intestine, and stomach. Therefore users are advised to apply caution in the use of this plant.

**1059** VALPROATE INHIBITS CEREBRAL GLUTAMINE UPTAKE AND OXIDATION: A CARBON 13 CELLULAR METABOLIC APPROACH APPLICABLE TO DRUG DEVELOPMENT.


Glutamine is both synthesized in astrocytes and metabolized in neurons. Our objective was to evaluate the brain slices and the cellular metabolic approach as tools for drug research and development. Slices prepared from rat brain hemispheres were incubated with 5 mM L-[3-13C]glutamine with 0 and 1 mM valproate, an antiepileptic drug. Substrate removal and product formation were measured by enzymatic and carbon 13 NMR methods. Fluxes through the enzymatic steps involved were calculated with an original mathematical model. In the absence of valproate, 3-13C-glutamine was used as substrate by glutaminase but glutamine synthesis also occurred in astrocytes. 3-13C-glutamate accumulation was also observed. GABA was found labeled mainly on its C3 (direct synthesis) and, to a very small extent, on its C2 (indirect synthesis). Accumulation of aspartate, labeled mainly on its C2 and C3, was also observed. Labeling of glutamate and glutamine on carbons other than their C3 reveals that synthesis of both amino acids occurred. Approximately half of the C3 of the 3-13C-glutamine removed was released as CO2. In the presence of valproate, 3-13C-glutamine removal was slightly inhibited. Aspartate accumulation and labeling on its C2 and C3 were decreased whereas GABA labeling on its C3 was increased by valproate. Valproate also diminished the production of 13CO2 but did not change the cellular ATP level. Flux calculations indicate that valproate slightly inhibited flux through glutaminase and caused an accumulation of GABA by the direct pathway. It also inhibited fluxes through succinate semialdehyde dehydrogenase plus alpha-ketoglutarate dehydrogenase, succinate dehydrogenase, fumarase, malic enzyme, aspartate aminotransferase and pyruvate carboxylase. By contrast, valproate did not alter fluxes through the other enzymes involved. It is concluded that the model used in vitro and the cellular metabolic approach employed are suitable for studying the beneficial and adverse interactions of drugs with brain energy and intermediary metabolism.

**1060** EFFECTS OF CAL-101, A SELECTIVE INHIBITOR OF THE CLASS I PI3K P110DELTA, ON LYMPHOCYTES IN SPEEN AND LYMPH NODES.

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One of the most frequently observed defects in human malignancies is the deregulation of the PI3K/Akt pathway, and several genetic and inhibition studies indicate that the PI3K isoforms play an important role in tumor growth. In contrast to the embryonic lethal p110α knockouts, mice with inactive p110δ kinase are viable and fertile with specific decreases in B-cell survival and function. CAL-101 is a potent and selective inhibitor of p110δ. In enzymatic and cell-based assays, CAL-101 was observed to have low nM potency against p110δ kinase with greater than 30-500 fold selectivity against other lipid kinases and greater than 10,000-fold selectivity against other kinases. CAL-101 inhibited B-cell receptor-stimulated B-cell proliferation with an IC50 of 6 nM. CAL-101 was assessed in a variety of in vitro assays and in vivo studies, including acute and subchronic GLP safety studies in rats and dogs. In 28 day non-clinical safety studies in rats, a wide therapeutic index was observed even at high plasma drug levels. In the rats, there was a dose-related decrease in secondary follicles of the lymph nodes and Peyer’s patches, a decrease in marginal zone lymphocytes of the spleen, and cortical lymphocyte depletion in the thymus as well as reversible decreases in total peripheral leukocytes. Immunohistochemistry showed a reversible, dose-related decrease in B-cell and, to a lesser extent and at higher doses, T-cell populations in lymphoid organs that corresponded with the microscopic findings in these tissues. These observations are consistent with the phenotype observed in p110δ knockout mice. CAL-101 demonstrated effects on target cells and tissues in vitro and in vivo consistent with the intended target and mode of action, and a wide therapeutic index has been established to enable clinical studies in normal volunteers, cancer and inflammatory diseases.

**1061** 6-MONTH TOXICITY STUDY OF IDURSULFASE VIA MONTHLY INTRATHECAL LUMBAR INJECTIONS AND WEEKLY IV INJECTIONS IN CYCLOMOLS MONKEYS.

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Mucopolysaccharidosis type II (MPS II or Hunter syndrome) is an X-linked disease caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS). Idursulfase, recombinant human IDS, is an IV enzyme replacement therapy for the somatic manifestations of MPS II. To treat the progressive central nervous system (CNS) disease which affects some patients, an intrathecal formulation of idursulfase (idursulfase-IT) is in development. Cycnomolys monkeys were dosed weekly with idursulfase (0.5 mg/kg, IV) and every 4 weeks with idursulfase-IT (3, 30, or 100 mg) for a period of 6 months via an intrathecal drug delivery device. The device and vehicle control groups received IV and IT injections on the same schedule. Body weights, clinical observations, neurological and physical examinations, clinical pathology, ECG, and ophthalmologic examinations were monitored. Six animals per group were necropsied at 6 months. In addition, 6 animals in the vehicle group and in the 3 and 100 mg IT groups were necropsied 4 weeks after the final IT dose. No clinical signs or gross lesions of the CNS were observed. As compared to controls, slight to mild increases in cellular infiltrates (mixtures of lymphocytes, neutrophils and eosinophils) in the meninges surrounding the brain and the spinal cord were noted during the microscopic examination, which correlated with transient increased WBC in the CSF. These changes had resolved after a 4 week recovery phase in the 3 mg idursulfase-IT group and were reduced in the 100 mg idursulfase-IT group. The meningeal infiltrates, which are commonly observed with IT dosing in general and with IT dosing of proteins in particular, were not associated with adverse morphologic changes in the brain or spinal cord, and did not result in clinical signs. The clinical, gross and microscopic findings indicated that idursulfase-IT was well tolerated at doses up to 100 mg.

**1062** HEPATOBILIARY TOXICITY OF A SMALL MOLECULE KINASE INHIBITOR IN THE DOG: INVESTIGATIVE ASSESSMENT.

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Oral administration of GEN-A, a small molecule kinase inhibitor, caused cholangiocellular hyperplasia/cholangiohepatitis and hepatocellular necrosis in beagle dogs above ~ 200 μM*hr plasma AUC levels, but not in rats at 10-fold higher plasma AUC levels. Investigational studies focused on the mechanism of toxicity causing the higher sensitivity of the dog, and the potential translatable of this toxicity to humans. Hepatocellular toxicity was investigated using primary rat, human and dog hepatocytes; the results indicate a slightly higher intrinsic sensitivity to cytoxicity in dog hepatocytes, and that the parent compound, rather than a metabolite, caused the cytoxicity. Two potential mechanisms of cholangiolar toxicity were explored: bile acid toxicity due to interference with bile acid transport/phospholipid transport into bile canaliculi (hepatic efflux transporters) and direct toxicity due to bile ductule compound accumulation. To assess the effect of GEN-A in hepatic efflux transporters, we measured the activity of BSEP, MDR1 and MDR2 transporter proteins using sandwich cultures of rat, human and dog hepatocytes. GEN-A did not clearly impair transporter function in any of the species examined, suggesting that inhibition of bile acid/phospholipid efflux are not likely to contribute to the hepatobiliary toxicity. In bile-cannulated studies, GEN-A reached 25-fold higher concentrations in dog bile (1.81 mM) compared to rat bile, despite a 50-fold higher plasma AUC in the rat compared to the dog, suggesting that GEN-A accumulation in dog bile duct may relate to the higher sensitivity of the dog to cholangiocellular toxicity. Efflux studies with GEN-A in hepatic sarcoma cultures are ongoing to further assess potential mechanisms of compound accumulation in dog bile and translational relevance to humans. The weight of evidence suggests that intrinsic hepatotoxicity and accumulation in dog bile might have contributed to the hepatobiliary toxicity of GEN-A in the beagle dog.
A number of atypical antipsychotics (AAPDs) are associated with unwanted side effect profiles such as weight gain and metabolic derangements which varies across the AAPDs. Treatment with olanzapine is associated with clinically significant weight gain and development of metabolic syndrome like phenotype, effects that increase with drug exposure, in patients. Treatment with aripiprazole is generally associated with minimal weight gain and a low risk of metabolic derangement. While olanzapine is a dopamine (D2)-receptor antagonist, aripiprazole is a partial agonist at the D2 receptor. The clinical benefits of AAPDs can be attributed in part to its activities on the dopamine and serotonergic receptors. Although the side effect profiles of the two AAPDs have been attributed to their activities on the histamine, cholinergic, muscarinic and alpha-adrenergic receptors, the underlying mechanism by which these compounds induce differential effect on obesity and metabolic syndrome is still largely unknown. The role of the hypothalamus in regulating food intake and obesity is well established. We therefore evaluated the molecular mechanisms in the hypothalamus that could potentially contribute to the differential metabolic side effects of the two AAPDs. We demonstrate that AMP activated kinase (AMPK), a master regulator in the hypothalamus, is activated by olanzapine but not aripiprazole. This activation was observed in hypothalamic slices treated ex vivo with compounds (200 – 500 nM) as well as in N46 hypothalamic cell line (5 – 5000 nM). Activation of AMPK was also observed in the hypothalamus following acute in vivo administration of olanzapine (3 mg/Kg) but not aripiprazole (30 mg/Kg). Using hypothalamic RNA from the acute in vivo study we demonstrate that olanzapine, but not aripiprazole regulate genes that stimulate the orexigenic pathway. Our study elucidates novel mechanism by which some AAPDs may impart unwanted side effects of obesity and metabolic syndrome.

Denufosol (denufosol tetrasodium) is a P2Y2 receptor agonist in Phase 3 clinical development as a potential pharmacological therapy for cystic fibrosis. The carcinogenicity of denufosol was detected tran- siently at high levels in bronchial alveolar lavage fluid after dosing, but did not accumulate in lung tissue. No significant or sustainable systemic exposure to denufosol was detected. In conclusion, no carcinogenic or adverse non-neoplastic effects were observed following daily inhalation administration of denufosol to Sprague-Dawley rats for a minimum of 104 consecutive weeks at total doses of 0, 13, 26, and 57 mg/kg body weight/day (males) and 0, 27, 53, and 108 mg/kg body weight/day (females). These high doses were set based on previously established maximum tolerated doses for each sex. Evaluations included clinical observations, body weight, food consumption, hematology, macroscopic observations at necropsy, histopathology, and plasma and lung toxicokinetics. There were no denufosol-related effects on mortality. Group mean body weight, body weight gain, and food consumption were reduced relative to controls in mid and high dose groups. No treatment-related changes in hematology or macroscopic observations were noted. There were no neoplastic microscopic changes related to denufosol in the respiratory system or other tissues or organs examined. Denufosol-related non-neoplastic changes included minimal to slight epithelial hypertrophy/hyperplasia in the larynx, trachea, and lung in the mid and high dose groups. These changes were not considered adverse based on the low severity and absence of associated degeneration, inflammation, neoplasia or unscheduled deaths. Denufosol was detected tran- siently at high levels in bronchial alveolar lavage fluid after dosing, but did not accumulate in lung tissue. No significant or sustainable systemic exposure to denufosol was detected. In conclusion, no carcinogenic or adverse non-neoplastic effects were observed following daily inhalation administration of denufosol to Sprague-Dawley rats for a minimum of 104 consecutive weeks at doses up to 57 mg/kg body weight/day (males) and 108 mg/kg body weight/day (females). These doses are approximately 6- to 12-times and 15- to 30-times greater than the clinical dose of denufosol normalized to body weight for children and adults, respectively.

Sibutramine is a novel anti-obesity drug, acts centrally by stimulating Serotonin (5-HT) and nor-adrenaline reuptake inhibition. It decreases body fat by reducing feed intake, enhancing satiety and increased thermogenesis. Although declared safe for human use, at few occasions it has been withdrawn from market due to some issues related to cardiac toxicity. Extensive information on the effect of sibutramine on body weight and food intake is available. In contrast, very sparse information is available about its toxicity. Therefore the present study was designed to assess the toxicological properties of sibutramine. Young healthy 6-8 weeks old Wistar rats were divided into four main and three toxicokinetic groups having 5 and 3 rats per sex per group respectively. Rats were gavaged daily with 0, 10, 30 and 100 mg/kg/day sibutramine for 7 days. Various toxicological parameters were studied during and at the end of the treatment period. Toxicokinetic analysis was performed on day 1 and 7. Mortality and clinical signs were observed at 100 mg/kg. Dose dependent decrease in body weight and food consumption was observed in sibutramine treated groups and was more evident during first three days of treatment. Significant decrease in absolute weight of spleen, thymus and increase in heart (females only) weights was recorded in 100 mg/kg sibutramine treated rats as compared to control. On macroscopic examination, reduction in abdominal fat mass, small size of spleen and thymus were recorded in rats treated with 100 mg/kg sibutramine. Microscopic examination revealed varying degree of atrophic changes in thymus and spleen at 100 mg/kg. Sibutramine was detected in plasma samples of treated animals on toxicokinetic analysis. In conclusion, there was no adverse effect in Wistar rats treated with sibutramine up to 30 mg/kg/day for 7 days.

The development of human therapeutics is severely compromised by three factors: lack of efficacy, unwanted toxicity and exploring cost. With toxicity remaining the leading cause for clinical attrition, risk assessment and drug development plans require urgent improvement. Here, we present an approach that is designed to support compound ranking in the hit-to-lead phase, aid lead selection, guide drug development planning, and facilitate decision making processes in the most cost-driving areas (e.g. GMP-manufacturing). We tested five exploratory compounds (MRZ52390, MRZ534743, MRZ51160, MRZ55451, MRZ57876)
along with six approved pharmaceuticals and five NIH-reference chemicals in an in vitro cytotoxicity screen using 3T3 fibroblasts according to NIH guidance No.01-4500. To further improve and validate the method, we added ATP-level as a second tos-endpoint. ATP-level is complementary to the first endpoint “metabolism” and addresses different toxicological aspects. The resulting logIC50 values of the reference compounds derived from both tos-endpoints were plotted against their ro- dent logLD50 values and the resulting regression line was used to estimate in vivo starting doses for acute toxicity studies of the exploratory compounds. The described in vitro-to-in vivo extrapolation then performed the dose titration test and improved the entire system’s predictive power. In summary, the advantages of this more comprehensive approach is (1) better estimation of in vivo starting doses for acute tox-studies, (2) enhanced risk assessment by addition of complementary tos-endpoint, (3) low compound usage, (4) generation of a basis for GLP/GMP manufacturing campaigns, and (5) for biology-driven compound ranking.

1069 INVESTIGATION OF LIVER X RECEPTOR PATHWAYS AS AN OFF-TARGET MECHANISM FOR INCREASED LIPID ACCUMULATION AND ADRENAL VACUOLATION IN THE RAT.

Liver X Receptors (LXRs) are ligand-activated transcription factors in the nuclear hormone receptor superfamily. Two isoforms of LXR (LXRα and LXRβ) have been identified with varying tissue distribution. The endogenous ligands of LXRs in- clude metabolites of cholesterol, such as the oxysterols 22(R)-hydroxycholesterol and 24(S)-hydroxysterol. LXRs regulate cholesterol homeostasis by transcrib- ing genes involved in cholesterol eflux (e.g. ABCA1), cholesterol storage (e.g. apoE, SREBP-1c), and metabolism (e.g. CYP7A1, StAR). Mice deficient in LXRα/β mice show increased lipid accumulation in the adrenal gland and a disruption of adrenal cholesterol homeostasis. In vivo safety studies in rats conducted during Lead Optimization identified an off-target dose dependent adrenal finding, characterized by increased adrenal cortical vacuolation and a corresponding increase in adrenal weights. Because of the similarity of the lesion to the phenotype of the LXRα(-/-) mice, studies were conducted to characterize the effect of the compound on LXR activity and LXR-dependent gene expression. We found that the compound weakly inhab- ited radioligand binding to LXR α as well as attenuated LXR-reporter activity in a cell-based functional assay. When challenged with compound, in vitro cultures of adrenal cortical cells showed increased lipid accumulation together with changes in gene expression consistent with perturbation of the LXR pathway and steroidogen- esis. These results suggest a potential off-target mechanism underlying the increased adrenal weights and vacuolation observed in vivo.

1070 MITOCHONDRIAL AND NEUROPATHIC TOXICITY OF THE ANTIBIOTIC LINEZOLID.
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Background: Linezolid blocks bacterial infection by inhibiting prokaryote protein synthesis. Long-term treatment has been associated with side effects, such as pri- mary mitochondrialopathies like hypertalactemia, anemia and peripheral neuropa- thy. Evolutionary similarity between mitochondrial and bacterial ribosomes and blockage of mitochondrial protein synthesis have been proposed to underlie toxic effects of linezolid therapy. Methods: We measured mitochondrial function, pro- tein synthesis, transcription and genetics on lymphocytes and monocytes of 8 pa- tients under long-term treatment with linezolid by using enzymatic assays, western blot, real-time PCR, haplotype determination and sequencing of the ribosome RNA where linezolid binds to mitochondrial ribosome (16SrRNA). Neuropathic analyses were performed on skin biopsies by staining of intraepidermal and dermal nerve fibers. Results: Mitochondrial enzyme activities and protein synthesis de- creased 49% and 84% respectively in patients after linezolid treatment, despite of a 197% increase on mitochondrial transcription. Two polymorphisms on mitochondri- al 16SrRNA gene were detected in three patients. All they presented the hap- logroup U5-defining m.3197T>C polymorphism and one of them m.1701T>C. Conclusions: Despite the dramatic increase of mitochondrial transcription, inhibi- tion of mitochondrial protein synthesis and function underlie side effects of long- term linezolid therapy. Polymorphisms on mitochondrial DNA could modulate the severity of linezolid toxicity and clinical outcome. Financial support: CIBERER.

1071 IDIOSYNCRASY IN TOXICOLOGY STUDIES.
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Idiosyncrasy, or variations in responses to stimuli by individuals, is an ancient idea dating back 2400 years to Hippocrates, but it is widely misunderstood. Concerning adverse responses to drugs, it has been characterized as rare, unpredictable, not dose-related, and seen in people but not in reasonable numbers of ordinary labora- tory animals. That belief may contribute to inhibiting research using animals to in- vestigate mechanisms of drug-induced liver injury. In humans, idiosyncratic re- sponses to the same dose-duration of the same drug is not necessarily rare, but occurs quite commonly in their responses to effects of drugs such as isoniazid, where a standard dose of 300 mg daily causes 20-30% of recipients to show serum alanine aminotransferase (ALT) elevations, although nearly all of them adapt and become tolerant of continuing the drug. Here we note inter-individual animal re- sponses in recent studies conducted by the NTP of known hepatotoxicants (inclu- ding bromobenzene, thiocarbamide, and galactosamine). Acute treatments were with four doses including those expected to cause mild or no liver injury. Fischer 344N male rats, 4 animals/group, were sacrificed at 6, 24, or 48 hours. Similarly, inter-in- dividual animal responses are documented in an NIEHS study where rats were sac- rificed at 6, 18, 24, and 48 hours after an acute treatment of acetaminophen (five dose groups). We also noted that these highly inbred rats, at the same dose and du- ration of toxicant compounds, showed obvious differences in response of degree of liver necrosis and serum enzyme elevation that appeared to be idiosyncratic, not rare, predictable, and dose-related. Inter-animal differences in ALT responses as great as 20 fold were observed after galactosamine or thiocarbamide, and as great as 70 fold after acetaminophen. The implications of this are that animal models are tractable for studies of various levels of hepatic injury and phenotypic idiosyncratic
1074 VALIDATION OF IMMUNOHISTOCHEMICAL STAINING IN TISSUE CROSS-RACTIVITY AND INVESTIGATIVE TOXICOLOGY STUDIES: LESSONS LEARNED FROM IMAGE ANALYSIS QUANTIFICATIONS.

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In our facility, antibodies used as research reagents or in development for human therapeutic use and the corresponding immunohistochemical staining procedures are subjected to a validation process prior to bringing into use for GLP tissue cross-reactivity and toxicology studies. Full validation consists of the determination of specificity, optimal concentration (C), precision (repeatability on triplicates), reproducibility (inter-day on three different days), linear range and dynamic range (from concentration without background noise to extinction of signal). The validation process is less exhaustive with antibodies for research use only. Each new staining technique is optimized manually and by automated instrument on frozen and fixed tissues. The most critical steps are determination of the optimal concentration and linear range using image analysis software to provide quantification of staining (stained surface area or number of stained cells) as a function of antibody concentration. Precision and reproducibility are calculated as coefficient of variation (%CV). Depending on the project, precision at [C] or 5×[C] %CV on triplicate sections are determined to be from 14% to 62%, while %CV reproducibility at these concentrations is between 15% and 44%, which is slightly better. Linear ranges were usually from 10 to more than 1000. Since in some cases, intra- and inter-run variability are more than 40%, a qualitative validation is sometimes the only way forward. High technical standards, reproducible section thickness, the use of digital slide acquisition and new approaches in image analysis can significantly improve intra-run variability. We provide an example where we improved the precision from 22 % to 14% at [C] and from 44% to 18% at 5×[C] using Gene® software. These results provide a framework by which analytical standards may be proposed for performance of immunohistochemistry assays with the highest quality.

1073 REFINED TECHNIQUES FOR COLLECTION OF SERIAL BLOOD SAMPLES FROM RODENTS AND NON-RODENTS FOR GENERATION OF TOXICOGENETIC DATA.


Use of dried blood spot (DBS) samples during pre-clinical safety assessment is a rapidly developing technique which also offers significant refinements in the way blood samples are collected from rodents and non-rodents together with a reduction in the number of rodents required for generation of toxicokinetic data. This poster presents refined blood sampling procedures for obtaining DBS samples from mice, rats, rabbits, dogs, minipigs and non-human primates. DBS samples were obtained from the tail vein of mice and rats either by needle prick or by insertion of a fine needle. As a result, serial blood samples (up to 12 within 24 hours) were collected without the need for anaesthesia or sacrifice. In addition, there was minimal bruising to the tails of both species after sampling. Multiple DBS samples were obtained from the marginal ear vein of rabbits. After sampling blood flow ceased within a few seconds of withdrawing the needle and there was minimal bruising at the sample site. In addition, external irritants to increase vein visibility were not required. Refinements in sampling from the non-human primate and dog were made by obtaining serial DBS samples from the cephalic, rather than the femoral or jugular vein. No occlusion was required to raise the cephalic vein and when the needle was withdrawn, blood flow ceased immediately. For minipigs, obtaining DBS samples by pricking the ear was a significant refinement compared to sampling from the jugular vein. Behavioural assessment clearly demonstrated these non-rodent species were more comfortable and showed less fear or aggression when the cephalic vein, or ear, was used for blood sample collection. Where bruising did develop, this was slight and had dissipated with 24 hours. On each occasion the DBS samples were collected into capillary tubes prior to spotting onto cards which were air dried and stored at room temperature until required for analysis.

1070 ALANINE AMINOTRANSFERASE (ALT) ELEVATIONS DURING ACETAMINOPHEN THERAPY IN OSTEOARTHRITIS (OA) PATIENTS ARE TRANSIENT AND NOT CLINICALLY MEANINGFUL.


Rationale: Reports of ALT elevations ≥3X upper limit of reference range (ULRR) in healthy volunteers given acetaminophen 4000mg/d for ≤2 weeks exist. We retrospectively analyzed ALT data collected within ~2 weeks of initiating therapy with the maximum recommended daily dose (MRDD) of acetaminophen (3900-4000mg/d) and determined elevated ALT and rates of resolution. Methods: 3 McNeil-sponsored, controlled OA trials met analysis requirements. Patients with elevated ALT at screening or baseline were excluded. Maximum ALT for each patient was stratified by degree of elevation. Resolution of ALT elevation was defined as an ALT ≤ULRR after last dose of acetaminophen. If ALT at study completion was lower than the maximum ALT observed during the trial but >ULRR, the ALT elevation was considered to be decreasing. Results: All trials had double-blind study designs, duration ≤12 weeks, and patients received the MRDD of acetaminophen. ALT remained within normal range throughout the initial 2 weeks for 376 (80.7%) of 466 patients. At some point during the initial 2 weeks, 90 (19.3%) patients had an ALT ≥ULRR; 21 (4.5%) had an ALT ≥3X ULRR; and 4 (0.9%) had an ALT ≥3X ULRR. No patient had an ALT ≥5X ULRR, or ≥3X ULRR with bilirubin ≥2X ULRR. Among the 90 patients with elevated ALT, ALT resolved for 62 (68.9%) and decreased for 19 (21.1%). No patient developed hepatic failure or hepatic dysfunction. Incidence of adverse events possibly of hepatic origin was similar between patients without and with elevated ALT during the initial 2 weeks of acetaminophen treatment. Conclusions: Fewer than 5% of OA patients taking the MRDD of acetaminophen for 2 weeks had an elevated ALT ≥3X ULRR. ALT elevations resolved or were decreasing during continued therapy in ≥95% of patients and were not accompanied by signs or symptoms suggestive of liver toxicity. These data show that ALT elevations occurring during ongoing acetaminophen treatment at the MRDD are transient and not clinically meaningful.

1075 EXOCRINE PANCREATIC TOXICITY INDUCED BY AN ARGinine-BASED iNOS INHIBITOR.

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Exocrine pancreatic toxicity was observed in a pre-clinical 4-week toxicity study in dogs with an arginine-based iNOS inhibitor. Dose-dependent vacuolation (mocchondrial swelling) and atrophy were the primary lesions observed. At high doses the atrophy was severe and encompassed virtually the entire tissue. Adverse effects were apparent only in the exocrine portion of the pancreas (acinar cells). Traditional serum biomarkers of pancreatic injury (amylase and lipase) did not reliably indicate the appearance or progression of the toxicity. The toxicity was species-specific, with dogs much more sensitive than rats. However, there was significant heterogeneity of the response in dogs, with approximately 50% of the animals demonstrating the lesion. Cytotoxicity assays in isolated rat and dog pancreatic acinar cells demonstrated that dog cells were more sensitive than rat cells to the test compound, although the compound was cytotoxic only at very high concentrations. Whole body autoradiography of animals exposed to radiolabeled compound demonstrated that accumulation occurred in both muscle and pancreas tissues. A search of the literature revealed that high doses of cationic amino acids (e.g. arginine and lysine) are associated with pancreatic injury. In summary, the combination of severe exocrine pancreatic toxicity, possibly due to accumulation of test compound in the target tissue, coupled with the lack of consistent biomarkers for these effects precluded further development of this compound.

1076 TOXICITY OF ARGinine-BASED iNOS INHIBITORS IS DUE TO ACCUMULATION OF HIGH LEVELS OF COMPOUND IN EXOCRINE PANCREATIC TISSUE.

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Mitochondrial swelling and atrophy occurred in the exocrine pancreas of dogs treated with an arginine-based iNOS inhibitor, SD-3651. A linear correlation between dose and pancreatic tissue burden was noted and, in multiple studies of vary-
ing duration, high tissue levels (≥400 μg/g) correlated with the appearance of pancreatic lesions. A series of in vitro and in vivo studies were carried out to confirm this hypothesis and identify a path forward. Rapid uptake of SD-5749 (the active component of SD-3661) into pancreatic acinar cells occurred via the y+ transport system, which normally targets cationic amino acids. An in vitro assay measuring uptake of radiolabeled SD-5749 into isolated dog pancreatic acinar cells was utilized to identify compounds that did not compete with SD-5749 for this cellular uptake pathway. Promising compounds were subsequently tested in a series of in vivo assays in dogs including 1) measurement of tissue accumulation following a single oral dose of 10 mg/kg, 2) an IV infusion study measuring plasma and pancreatic tissue levels after 4 and 8 hr, and 3) a definitive 7-day study at an exposure equivalent to SD-5749 that produced pancreatic toxicity. The in vitro cellular uptake assay proved predictive of in vivo tissue accumulation and toxicity. Two compounds with reduced in vitro cellular uptake were identified that resulted in greatly reduced pancreas tissue levels and lack of mitochondrial swelling or atrophy in the 7-day dog study. In contrast, a third molecule, which was similar to SD-5749 in the acinar cell uptake assay, produced a very high pancreatic tissue burden and caused mitochondrial swelling and atrophy. These results validated the "pancreatic tissue burden" hypothesis and led to the identification of compounds with reduced tendency for accumulation in pancreatic tissue.

**1079 A CELLULAR SYSTEMS BIOLOGY APPROACH TO DISCRIMINATE HEPATIC TOXICITY WITHIN STRUCTURAL CLASSES.**

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Despite an aggressive campaign in the pharmaceutical industry to identify liver liability earlier in the development process, clinical hepatotoxicity continues to be an important cause of late stage drugs failures. The Cellular Systems Biology approach from Cellumen captures the response of 24 or more phenotypic and mechanistic biomarkers in relevant cell types at multiple doses and time points. The responses comprise a profile which can be compared to Cellumen’s drug profile database to identify correlations with known in vivo effects and to classify or rank order the risk of liver injury. This technology was applied to 5 pairs of compounds that met the following criteria: (a) silent in animal safety studies, (b) structural analogs, (c) withdrawn from market due to unacceptable hepatotoxicity, and (d) difficult to discriminate toxic from non-toxic with existing technology. The sets include the hepatotoxic compounds: Trovofloxacin, Tolcapone, Bromfenac, Ibuprofen, Nefazodone matched to the clinically non-hepatotoxic or reduced hepatotoxic compounds: Lomefloxacin, Entacapone, Diclofenac, Ibuprofen and Trazodone. The matched sets were tested at 3 time points and 10 doses in a HepG2 panel of 10 assays, and in a rat primary hepatocyte panel of 8 assays. The Cellumen HepG2 panel includes features to measure oxidative stress, stress kinase activity, DNA damage, mitochondrial function, cell cycle modulation, nuclear size and cell loss. The Cellumen rat hepatocyte panel includes markers to measure ER stress/DNA damage, apoptosis, phospholipidosis, steatosis, mitochondrial function, DNA fragmentation, nuclear size and cell loss. Three computational classifiers were used to score the compounds for hepatotoxicity based on their profiles. One classifier, which combines the profiles of both panels, out-performed a HepG2-only and a rat hepatocyte-only classifier, correctly ranking 4 out of 5 toxic compounds. These studies indicate the value of Cellumen's Cellular Systems Biology approach to identify compounds of high risk to elicit clinical hepatotoxicity.

**1080 OPTIMISING CELL MODELS FOR DETECTING DRUG-INDUCED MITOCHONDRIAL DYSFUNCTION.**

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Drug-induced mitochondrial dysfunction is increasingly implicated in the etiology of organ toxicities, underscoring the need for predictive preclinical models. Cell lines, such as HepG2, are typically grown at high concentrations of glucose where they generate ATP via glycolysis. Such cells tolerate mitochondrial toxins and so fail to detect drugs with mitochondrial liabilities. When glucose is replaced by galactose, HepG2 cells shift their bioenergetic poise towards oxidative phosphorylation and so become susceptible to mitochondrial toxins I. To determine how such cells compare to primary hepatocytes, we monitored oxygen consumption and found that while HepG2’s grown in galactose have higher respiration than cells grown in glucose, the oxygen cost of activation of primary hepatocytes was ~6-fold higher. Flow cytometry was also used to compare the mitochondrial membrane potential (ΔΨm) and the proportion of dead cells in each population. In accord with the respiration data, HepG2 cells grown in galactose have more intense NAO staining indicative of increased cardiolipin and they maintain higher ΔΨm than either population of HepG2’s. Treatment with oligomycin yielded a dose dependent loss of mitochondrial membrane potential and cytotoxicity in the HepG2 cells grown in galactose, which was not observed in cells grown in glucose. Similar differences were seen with single doses of antimycin and rotenone; however no difference was observed when cells were treated with FCCP or KCN. In all instances, primary hepatocytes were more susceptible to these toxins than either population of HepG2 cells. The data indicate that growing HepG2 cells in galactose did increase respiration and their susceptibility to three out of five of the mitochondrial toxins evaluated here. However, primary hepatocytes are more aerobically poised and than the highest concentration evaluated (100 μM). Furthermore, the panel demonstrates the importance of cardiac organ specificity, as several known cardiototoxicants including the antihistamine terfenadine activated more toxicity biomarkers in this cardiac panel when compared to a hepatic model. Taken together, these results demonstrate this Cellular Systems Biology cardiototoxicity panel from Cellumen is both selective and sensitive in accurately discriminating safe and toxic compounds in cardiac tissue as well as elucidating the potential mechanisms responsible for cardiototoxicity.
hence more physiologically relevant predictors of in vivo toxicity, which recommends them as the in vitro model of choice for pre-clinical toxicity testing. Reference 1. Marroquin et al., 2007 "Tox Sci" 97

**1081 IMPACT AND FREQUENCY OF DIFFERENT TOXICITIES THROUGHOUT THE PHARMACEUTICAL LIFE CYCLE.**

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Prioritisation of safety assessment resources according to impact and prevalence of toxicities is often based on opinion, anecdotal examples, recent issues with specific projects, and regulatory focus. Evidence-based decision-making requires reliable data on drug-induced toxicities across a range of organs/categories. We have collated information from published reviews across the following categories of toxicity: cardiovascular (CV), nervous system (N), respiratory (RES), gastrointestinal (GI), renal (REN), hepatotoxic (HEP), musculoskeletal (MSK), haematology/hormone (HBM), immune and photosensitivity (IMT/PTS), reprotox (RTX), genetic tox (GTX), and carcinogenicity (CNG). Some datasets relate to frequency of candidate/withdrawn drugs associated with the toxicity, but contain data on previous occurrence of the toxicity in subjects/patients, as follows. 1. Nonclinical attrition (based on 88 CDs stopped): CV (27%) > N (14%) > RTX (13%) > HBM; IMT/PTS (7%) > GTX (5%) > MSK (4%) > GI; CNG (3%) > REN/RESP (2% each). 2. Phase I serious ADRs (based on 43 events in 1,013 subjects): N (28%) > GI (23%) > IMT/PTS (17%) > CV (9%) > HBM (2%). 3. Clinical development – causes of attrition (based on 82 CDs stopped): N; CV; HBM (21%) > IMT/PTS (13%) > RES (11%) > CV (9%) > GI (8%) > REN (4%). 4. Marketed drugs - serious ADRs – causes of attrition (based on 88 CDs stopped): CV (27%) > N (14%); RTX (13% > HBM; IMT/PTS (7% > GTX (5%) > HBM (2%); RES (2%). 5. Marketed drugs - serious ADRs (based on 21,298 patients): N (39%) > IMT/PTS (34%) > CV (15%) > GI (14%) > HBM (10%) > RES (8%) > MSK (3%) > REN (2%). 6. Marketed drugs - serious ADRs (based on 43 events in 1,015 subjects): N (28%) > GI (23%) > IMT/PTS (21%) > CV (9%) > HBM (2%). 7. Marketed drugs - serious ADRs (based on 82 CDs stopped): CV (27%) > N (14%); RTX (13% > HBM; IMT/PTS (7% > GTX (5%) > HBM (2%); RES (2%). These data require cautious interpretation, but are a first step to assessing frequency and impact of different drug-induced adverse effects throughout drug discovery, development and marketing. Sources: 1. ADD (2006) 1:53-65; 2. EUCA (1998) 54:13-20; 3. RegTP (2000) 32:56-67; 4. JAMA (2000) 296:1858-66; 5. DDT (2009) 14:162-67.

**1082 THE USE OF IMPLANTATION OF TRANSPONDERS IN TG.RASH2 MODELS FOR CARCINOGENICITY ASSESSMENT: THE USE OF SUBCUTANEOUS IMPLANTATION OF TRANSPONDERS IN TG.RASH2 MODELS FOR CARCINOGENICITY ASSESSMENT.**

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Subcutaneous implantation of transponders is a commonly used method for animal identification in preclinical studies. Sufficient toxicity data is available to support the use of these implants in various conventionally used rodent strains that are commonly used in toxicology studies. However, robust data is not available to support the use subcutaneous transponders in the emerging transgenic models, such as the Tg.rash2 mice. This mouse model is commonly used in 26-week carcinogenicity studies, and the objective of this study is to determine if implantation of transponders increases the incidence of tumors in this model over a 26-week period. Forty Tg.rash2 mice per sex (8x2 weeks) were implanted with microchip transponder subcutaneously, and an additional 20 males and 25 females were used as a trocar control group (not implanted with the transponder, but subjected to subcutaneous injection procedure). Mice were necropsied 26 weeks post-implantation of the transponder. The primary endpoints of the study were macroscopic and microscopic analyses of implantation (injection) sites. No gross lesions were noted at the skin site of injection (SOI) in transponder-implanted or trocar control mice at necropsy. The skin SOI trocar control mice was considered to be within normal limits for all mice. Histopathological examination revealed that the transponder-implanted skin SOI was within normal limits for 14/40 males and 12/40 females. In the transponder-implanted animals, skin cavity formation was noted in the dermis of 25/40 male and 27/40 female mice, and connective tissue surrounding these cavities was compressed in 24/40 males and 26/40 females. Fibrosis, of minimal to mild intensity, was noted in the dermis directly surrounding the cavities in some of these mice. No tumors were observed at the SOI in any of the mice at all groups. It is therefore, concluded that the use of transponders in the Tg.rash2 mice would not negatively impact the outcome of a 26-week carcinogenicity study.

**1083 METHODS FOR SUCCESSFUL CONDUCT OF CHRONIC TOXICOLOGY INTRAVENOUS TAIL VEIN INJECTION STUDIES IN RATS.**


Performing rodent chronic toxicology studies via daily intravenous injection can be challenging. Appropriate experimental procedures as well as consideration for the dose volume and properties of the formulation to be administered are required. Studies were conducted at PCS-MTL using phosphate buffered saline or 0.9% saline administered by daily slow bolus intravenous injection (tail vein) for up to 182 consecutive days. The rats were between Day 21 post partum and 7 weeks old at the start of dosing. Animals were restrained using the Horizontal Cylinder (claw type) device as per PCS-MTL standard operating procedures. Rats were placed in the restrainer and adjustments made as necessary so as not to restrict chest expansion for breathing. To facilitate injection, a heating device, the Hot PadTM, was used to dilate the caudal vein. If the injection site was compromised, dosing was suspended for up to 6 consecutive days to allow sufficient recovery. To minimize the severity of any skin lesion along the tail and/or to aid recovery at the injection site, calamine lotion was occasionally used as an antipruritic agent. Dosing holidays were required for 12% of the animals (26 rats from a total of 210 animals) with a maximum of 19 non-consecutive days (10% of the total number of doses) without dose administration for any given animal over the 182 days of dosing. Damage to an injection site such that dosing was no longer possible requiring that the animal was removed from the study occurred in 3% of animals. Two animals necessitated surgical intervention for amputation of a portion of the tail; however, dosing was still successfully maintained. In conclusion, it was demonstrated that with appropriate care of the injection site, rats starting as young as Day 21 post partum can successfully be dosed by daily intravenous injection via the tail vein for up to 26 consecutive weeks.

**1084 ATRIOVENTRICULAR (AV) VALVULAR INJURY CAUSED BY A VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (VEGFR) INHIBITOR IN RATS APPEARS TO BE RODENT SPECIFIC.**

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VEGFR inhibition is a useful therapeutic strategy for treatment of a variety of cancer types, but has also been associated with a battery of unintended effects. In a rat 6 month oral toxicity study with a VEGFR inhibitor, dose-dependent increases in incidence and/or severity of AV valve thickening and hypercellularity were noted beginning at 3 months at doses of 20, 110, 200/160 mg/kg/day, which progressed to a valvulopathy characterized by endothelial loss and fibrin deposition (with or without proliferation of mesenchymal cells) in some males at 110 and 200/160 mg/day. Conversely, through 1 year of daily dosing with exposures overlapping those noted in rats, monkeys showed no evidence of valvular injury, including no evidence of echocardiograms, clinical biomarkers of cardiac injury or histopathologic effects on the AV valves. In separate telemetry studies, rats had a robust increase (up to 22 mmHg) in blood pressure (BP) following treatment with the VEGFR inhibitor while there was only a minimal increase in BP in monkeys, generally within normal fluctuation of controls. Detailed transcriptional examination of right and left AV valves in the rat heart, upstream of lesion development (ie, after 3 days and 1 month of dosing), failed to demonstrate a direct mechanistic based effect, but did suggest effects on inflammatory and extracellular matrix related transcripts. It has been reported that rodents have a spontaneous age-related endocardial myxoma-like change in heart valves that is similar to some cases of drug induced valvulopathy. Based on these data, the valvulopathy induced by this VEGFR inhibitor appears to be secondary to sustained increases in BP and a drug-related reduced ability for valvular repair that exacerbated a rodent-specific predisposition to valvular injury with little relevance to higher-order species, including humans.

**1085 COMBINATIONAL TREATMENT OF GAP JUNCTIONAL ACTIVATOR AND TAMOXIFEN IN BREAST CANCER CELLS.**

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Tamoxifen is a drug of choice for endocrine-responsive breast tumor patients. However, tamoxifen resistance has become a major concern for the treatment of breast cancer. Combinational therapies of tamoxifen and different drugs are being
frequently studied. In the current study, we tested the efficacy of substituted quinolines (code name = PQ1) in combination with tamoxifen in T47D cells. Previous studies conducted by our group shows that PQ1 is a gap junctional activator. Gap junctions are intercellular channels allowing the passage of small molecules from one cell to another. Loss of gap junctional intercellular communication has been observed in different kinds of cancer. Therefore, in our study we proposed to determine the combinational efficacy of PQ1 and tamoxifen on breast cancer. The results show that combinational treatment of PQ1 and tamoxifen cause a 55% decrease in the colony growth and compared to control (10 μM tamoxifen and PQ1 200 nM or 500 nM resulted in only 16% cell viability compared to controls at 48 hr in T47D cells by MTT assay. We found a significant increase in BAX protein at 1 hr in the presence of 500 nM PQ1 alone, 10 μM tamoxifen alone and combination of PQ1 and tamoxifen. A 2-fold increase was observed in active caspase 3 in the presence of combinational treatment of 10 μM tamoxifen and 200 or 500 nM PQ1. Also, flow cytometric analysis showed a 50% increase in the number of apoptotic cells in the presence of combination of tamoxifen and PQ1 compared to the control. Furthermore, the results showed that combinational treatment of tamoxifen and PQ1 significantly reduces the expression of survivin in T47D cells. In conclusion, the present study demonstrates that combinational treatment of tamoxifen and PQ1 (gap junctional activator) can be used to potentiate apoptosis of T47D human breast cancer cells. Thus, gap junctional activator, PQ1, could potentially alter either the length or dose of tamoxifen clinically used for breast cancer patients.

8086 ALTERATION OF CYTCHOME P450 GENE EXPRESSION IN THE KIDNEY AND LIVER OF MALE SPRAGUE-DAWLEY RATS BY ACUTE DOXORUBICIN TOXICITY.

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Doxorubicin (DOX) is a potent anti-neoplastic antibiotic used to treat a variety of malignancies; however, its use is limited by significant cardiotoxicity, nephrotoxicity, and hepatotoxicity. We have previously shown that DOX (doxorubicin) induces several cardiac cytochrome P450 (CYP) enzymes with subsequent alteration in CYP-mediated arachidonic acid metabolism. CYP-mediated arachidonic acid metabolites play pivotal roles in the heart, kidney, and liver physiology. Nevertheless, the effect of acute DOX toxicity on the expression of renal and hepatic CYP genes was not examined previously. Therefore, in the current study, we have investigated the effect of acute DOX toxicity on CYP gene expression in the kidney and the liver of male Sprague Dawley rats. Acute DOX toxicity was induced by a single intraperitoneal injection of 15 mg/kg of the drug. After 24 hours, the kidney and the liver were harvested and the expression of different CYP genes was determined by real time-PCR. Our results showed that acute DOX toxicity caused a significant induction of CYP1B1 and CYP4A5 in both the kidney and the liver. However, CYP2E1, CYP4A1, CYP4A2, and CYP4F1 were significantly induced in the kidney but not in the liver of DOX-treated rats. On the other hand, CYP2C11 gene expression was significantly inhibited in both the kidney and the liver, whereas CYP2B1 and CYP2B2 were inhibited significantly in the liver but not in the kidney of DOX-treated rats. The expression of CYP1A1, CYP4F4, CYP4F5, and CYP4F6 was not significantly altered in both the kidney and liver. In conclusion, acute DOX toxicity alters the expression of several CYP genes in an organ-specific manner. The changes in CYP gene expression may result in altered arachidonic acid metabolism. In addition, inhibition of several hepatic CYP enzymes may account for drug interactions with DOX due to inhibition of CYP-mediated drug metabolism. (This work was supported by the Heart and Stroke Foundation of Alberta, NW7, and Nunavut).

8087 REACTIVE METABOLITES OF BISPHENOL A FORMED BY RAT LIVER MICROSOMES AND TRAPPED BY DANSYL GLUTATHIONE.


Bisphenol A (BPA) is an environmental contaminant that has attracted considerable concern for its possible role as an endocrine disruptor of estrogen action. However, metabolism-derived toxicity of BPA is inadequately explored. We examined metabolism of BPA with rat liver microsomes (Mr) by trapping of possible reactive metabolites with dansyl glutathione (dGSH). Experimental procedures: BPA was incubated with 5 different Mr: β-Naphthoflavone-treated (NF), phenobarbital-treated (PB), pregnenolone 16α-carbonitrile-treated (PCN), vehicle-treated male (CM), and vehicle-treated female (CF), which represent the following P450s respectively: 1A1/2; 2B1/2; 3A1/2; 2C11, 2C13, and 3A2; and 2C12. The incubations were performed in phosphate buffer pH 7.4 at 37°C in the presence of NADPH and dGSH as a trapping agent. At the end of the incubation the reactions were terminated with 2 volumes of concentrated DTT in methanol. Mr were extracted with the most abundant adducts, BPA and phenol BPA, followed Michaelis-Menten kinetics. The 5-hydroxy metabolite was found unstable and formed at the lowest rate, while 4-isopropyldiol adduct peak was incompletely resolved from dGSH, hence, its kinetics could not be reliably determined at this time. Conclusion: Four reactive metabolites of BPA were formed by rat liver P450 enzymes in the order of decreasing activity: 2B1/2, 2C11, 3A1, 1A1/2.

8088 A KEY ROLE FOR CYP3A4 IN MDMA (ECSTASY) -INDUCED HEPATOTOXICITY AND IMPLICATIONS FOR ACUTE MDMA TOXICITY TREATMENT.

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Over the last years, an increasing number of reports on adverse, toxic effects after recreational use of MDMA (3,4-methylenedioxymethamphetamine, Ecstasy) have been published in the literature. Large interindividual variations in susceptibility toward MDMA (hepato)toxicity have been observed, which can largely be attributed to differences in both phases I and II metabolism. We have used the human liver epithelial (THLE) cell line transfected with a single cytochrome P450 to study the implications of metabolism on MDMA toxicity. Inhibition of the phase II enzymes catechol-O-methyltransferase (COMT) and especially glutathione (GSH) significantly increased MDMA toxicity after CYP3A4 and CYP2D6-mediated MDMA metabolism by about 50% and 20%, respectively. This indicates a major detoxification role for GSH in cells after CYP3A4 or CYP2D6-mediated metabolism of a physiologically relevant concentration of MDMA. These results were confirmed by experiments showing a 40% reduction of GSH level after MDMA exposure in THLE-CYP3A4, but not THLE-CYP2D6 cells. These data suggest that CYP3A4 is the main enzyme responsible for cytotoxic metabolism formation and GSH depletion after MDMA exposure, which might lead to hepatotoxic effects. CYP3A4 is also a major enzyme involved in the metabolism of therapeutic drugs. The potential interaction of uncontrolled use of MDMA with these prescription drugs, e.g. to control psychiatric disorders or in the treatment of acute clinical signs in MDMA toxicity, is of relevant concern. Moreover, many of these pharamaca can induce CYP3A4 via activation of the pregnane X receptor (PXR) thus potentially increasing MDMA-mediated toxicity. Further studies are being performed in our laboratory to determine this potential drug-drug interaction through PXR-mediated CYP3A4 expression using a reporter gene assay.

8089 THE MITOCHONDRIAL TRANSPORTER ABCB6 REGULATES CYTCHOME P450 ENZYME EXPRESSION.

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Cytochrome P450 (CYP450) enzymes belong to a superfamily of hemoprotein that play a pivotal role in the metabolism and clearance of various xenobiotics and environmental toxins. An important feature of CYP450 enzymes is an important structural module for CYP450 and is essential for its activity. Further, haem is also known to regulate the expression of CYP450 enzymes. In this study we investigated whether the mitochondrial half transporter ABCB6, which regulates cellular haem biosynthesis and cellular haemoprotein pool, plays a role in the expression and/or function of CYP450s. To investigate this question, we developed mice deficient in Abcb6 (Abcb6+/- mice) and evaluated the expression and activity of two liver specific CYP450s: Cyp3a11 and Cyp2c1. These mice demonstrate decreased porphyrin levels compared to Abcb6+/- mice (50% decrease in porphyrin levels in Abcb6+/- mice compared to Abcb6 +/+ mice). Interestingly, in spite of decreased porphyrin levels, we found thioether expression of both Cyp3a11 and Cyp2c1 was induced in Abcb6 +/- mice. However, this increase in expression did not translate into increased activity when tested with substrates specific for Cyp3a11 (midazolam) and Cyp2c1 (chlorozoxone). Cellular synthesis of CYP450s is thought to be tightly coordinated with haem synthesis to sustain adequate supply of haem for newly syn-
thethesized appoproteins, so we tested whether prototypical inducers of CYP450 expression regulated Alcb6 expression. We found that both pregnenolone-16α-carbonitrile (PCN) and 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP), two well known inducers of CYP450, induced ABCB6 expression and that this effect is mediated by the nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR). In summary these findings suggest that prototypical CYP450 inducers regulate Alcb6 expression and altered Alcb6 expression might play a role in the metabolism and disposition of drugs and environmental chemicals, and may affect drug clearance and drug toxicity.

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ST. JOHN’S WORT REDUCES TRIBROMOETHANOL-INDUCED SLEEP TIMES IN HUMAN-SXR TRANSGENIC MICE.
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Saint John’s wort (SJW) has been reported to reduce the efficacy of some drugs taken concurrently leading to adverse health outcomes in human patients. The mechanism underlying these interactions is thought to involve induction of cytochrome P450 3A (CYP3A), an enzyme responsible for the metabolism of approximately 50% of prescription drugs. Transcriptional regulation of CYP3A is governed, in part, by the steroid/ xenobiotic receptor (SXR), a ligand-activated transcription factor which has differential ligand specificity between mice and humans. To further study CYP3A regulation by SJW, transgenic mice expressing the human SXR gene (hSXR mice) were used. Commercially available SJW extracts were tested. hSXR mice were treated with vehicle (control) or SJW extract at 5 ml/Kg daily for seven days. On the eighth day, all animals were anesthetized with tribromoethanol (TBE). The duration of TBE-induced anesthesia (sleep) has been shown to correlate inversely with CYP3A11 mRNA levels. Two of the five SJW formulations caused a significant decrease in TBE-induced sleep times by up to 25% (p < 0.05). Daily treatment with rilpamipin, a known ligand of the hSXR, at 10 mg/Kg/day caused a similar decrease in TBE-induced sleep times. Real-time RT-PCR analysis of liver mRNA levels revealed no significant differences in CYP3A11 gene expression between vehicle-, SJW- and rilpamipin-treated mice. These results suggest that elevated CYP3A11 mRNA levels do not persist after exposure to inducing agents in this model.

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EXPRESSION OF CYP3A GENES IN THE MICE FED CHRONICALLY WITH ETHANOL.
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Pregnane X receptor (PXR) belongs to the superfamily I12 of nuclear receptors that coordinate protective hepatic responses to potentially toxic stimuli. It is primarily expressed in the liver and intestine. Activation of PXR stimulates the transcription of genes involved in the xenobiotics metabolism including cytochrome P4503A (CYP3A). According to our published microarray data, long-term administration of the ICR mice with ethanol increased the expression of PXR as well as a series of genes known to be regulated by it. To explore the role of PXR on the ethanol-induced Cyp3a11 expression, ethanol was administrated by feeding the standard Lieber-DeCarli diet for 4 weeks, of which 36% of total calories were supplied from ethanol. Long-term administration of ethanol significantly increased the expression of PXR mRNA and protein. Ethanol feeding also increased the nuclear localization of PXR. Ethanol increased the binding of PXR to the response element of Cyp3a11 promoter in a time-dependent manner. Consequently, the expression of PXR target genes was increased such as Cyp3a1, Cyp2b10 and CD36. The possible mechanisms of PXR-mediated gene expression by ethanol is discussed. Considering the effects of ethanol on the CYP gene expression, care should be taken when the therapeutic drugs metabolized by CYP3A were used in the patients who often consume alcohol.

1092
CHIPPING THE CISTROME OF PXR IN MOUSE LIVER.
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The pregnane X receptor (PXR) is a key regulator of xenobiotic metabolism and disposition. However, little is known about PXR DNA binding signatures in vivo, or how PXR regulates direct target genes on a genome-wide scale. Therefore, we have generated a roadmap of hepatic PXR binding signatures in the entire mouse genome in control and PXR-activated conditions (ChIP-Seq). The most frequent PXR DNA-binding motif is the AGGTCA-like direct repeat with a 4bp spacer (DR-4). Surprisingly, there are also high motif occurrences with spacers of a periodicity of 3bp, forming a novel DR-(5n+4) DNA structural configuration for PXR-binding (n=0,1,2,...,20), challenging the existing paradigm that DR-3 and ER-6 are more common response elements for PXR. Interestingly, PXR binding overlaps with the epigenetic mark for gene activation (histone-H3K4-dimethylation), but not with epigenetic marks for gene suppression (DNA methylation or histone-H3K27-tri-methylation) (ChIP-on-chip), indicating a permissive chromatin environment pre-defines PXR DNA binding signatures. After administering a PXR agonist, both the induction and suppression of most PXR-direct target genes correlate with increased PXR binding (microarray). Whereas the induced gene battery is mainly involved in drug metabolism and cell proliferation, the suppressed gene battery is important for amino acid and carbohydrate metabolism. Specifically, increased PXR binding triggers the trans-activation of critical drug-metabolizing enzymes and transporters, and the mRNA induction of these genes is blocked in PXR-null mice. In conclusion, we have provided the first in vivo evidence of PXR DNA binding signatures in mouse liver, discovered a novel DNA structural configuration for PXR binding, identified novel PXR direct target genes and critical epigenetic cofactors that pre-define the transcription potential of the PXR cisrome, paving the way for predicting and further understanding the multifaceted roles of PXR in mediating the physiological and pharmacological responses in humans. (Supported by NIH grants ES009716, ES096649, ES013714, DK081461, RR021940, RR016475, and NICHHD002528)

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QUANTIFICATION OF P450 CYP3A ACTIVITIES IN TWELVE ORGANS OF A CYNOMOLGUS MONKEY.
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As part of our overall objective to develop approaches for the evaluation of organ-specific xenobiotic toxicity, we have initiated a research program to characterize drug metabolizing enzyme activities in hepatic and nonhepatic organs in humans and preclinical animal species. We report here the quantification of P450 CYP3A activity of the adrenal gland, brain, heart, kidney, liver, lung, pancreas, skeletal muscle, intestinal mucosa, spinal cord, spleen and testis from a male Cynomolgus monkey. Post-mitochondrial supernatants (PMS) were prepared from the organs by homogenizing each organ in 1x weight of Tris.HCl buffer (pH 7.5) containing 1.5 mM EDTA and 150 mM KCl, followed by centrifugations at 1000 x g and at 10,000 x g. CYP3A activity of the PMS from the various organs was quantified using a CYP3A-specific substrate, luciferin-IPA (LIPA). LIPA is metabolized by CYP3A to luciferin which can be quantified by luminescence using an ATP-luciferase based detection reagent. The PMS from the various organs were incubated at 0.5 mg/ml PMS protein, with 1 mM NADPH, and 2 µM of LIPA. The incubation times were 30 and 60 minutes. Time-dependent LIPA metabolism to luciferin was detected for PMS from all organs, with the highest activity detected in the liver (6.7 pmol/mg/min protein), followed by intestine mucosa (0.20 pmol/min/mg protein), and lungs (0.03 pmol/min/mg protein). The remaining organs had substantially lower but detectable CYP3A activities, ranging from 0.01 pmol/mg/min protein (adrenal gland) to 0.005 pmol/min/mg protein (testis). The results show that CYP3A activity, although mainly located in the liver, is universally present in the nonhepatic organs studied and may contribute to organ-specific toxicity of xenobiotics which are substrates of this P450 isoform. Our results here represent the first to report on the distribution of CYP3A activity in the twelve organs in a Cynomolgus monkey.
Bioluminescent substrates are proluciferins that are converted by the enzymes of interest to a luciferin that is detected in a bioluminescent reaction with luciferase, correlating light output with target enzyme activity. Out of 21 human CYP enzymes luciferin-IPA only showed activity with the CYP3A subfamily, having 14 and 161 fold selectivity for CYP3A4 over CYP3A5 and CYP3A7, respectively. Cell free luciferin-IPA enzyme assays were readily configured for IC50 determinations with either recombinant CYP3A4 or human liver microsomes. CYP3A4 induction was measured in rapid, 96-well, human hepatocyte assays where activity was increased by known CYP3A4 inducers. The luciferin-IPA/CYP3A4 hepatocyte assay was also configured in various multiplex applications that provided additional measurements from single wells including CYP3A4 transcriptional activity, CYP1A enzyme activity and cell viability. While luciferin-IPA/CYP3A4 induction and inhibition data was virtually identical to data from conventional CYP3A4 testosterone 6-beta hydroxylation assays, the luminescent assays were simpler, more sensitive and substantially quicker. Luciferin-IPA assays enable early ADME screening with high throughput and rapid turn around times.

**1095** EFFECTS OF P450 INHIBITOR, 1-AMINOENBONTZIPRAZOLE (ABT), ON THE CYTOTOXICITY OF MODEL HEPATOTOXICANTS IN HUMAN HEPATOCYTES.

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The current study was designed to define the roles of P450-mediated metabolism in the in vitro cytotoxicity of the hepatotoxicants acetaminophen (Ap), cyclophosphamide (Cp), amiodarone (Am), tamoxifen (Tx), cadmium (Cd) and aflatoxin B1 (Af) in human hepatocytes, using a non-specific, mechanism-based P450 inhibitor, ABT. Human hepatocytes were plated at a cell density of 20,000 cells/well in 96-well collagen-coated plates for 2 hours for cell attachment, followed by a pre-treatment with medium with or without 500 μM ABT for 30 min. The cells were then treated with multiple concentrations of hepatotoxicants alone or in presence of ABT for a period of 6 and 24 hrs in either 95% air-5% CO2 (air) or 95% O2-5% CO2 (carbogen). Cell viability was assessed by measuring cellular ATP content. After the 6-hour treatment, a decrease in cell viability was observed at higher concentrations of Cd, Tx, Am, Ap, in air and carbogen, while no cytotoxicity was observed for Cp. A small but significant decrease in hepatocyte viability was observed for Af in carbogen but not in air. ABT decreased the cytotoxicity of Af (in carbogen) but not that for the other hepatotoxins. Cytotoxicity for the hepatotoxicants was more pronounced under 24 hr of treatment. ABT had no apparent effects on the cytotoxicity of Ap, Cd, Tx and Am in air and in carbogen. ABT significantly decreased the cytotoxicity of Af, with the protection being more prominent in carbogen. ABT had no effects on Cp mediated toxicity in carbogen but significantly potentiated its cytotoxicity in air. The data with Af is consistent with its P450-mediated metabolism in toxic metabolites. The higher protective effects of ABT for Af in carbogen may be due to its higher rate of metabolism in this atmosphere, thereby leading to more P450 inactivation. The reasons for unexpected results with Cp are yet to be determined. The results suggest that human hepatocytes, used in combination with metabolic inhibitors, may aid in defining the roles of xenobiotic metabolism in hepatotoxicity.

**1096** PREDICTIONS OF P450 BIOACTIVATION OF TAMOXIFEN ARE IMPROVED BY NEW COMPUTATIONAL SUBSTRATE-ENZYME MODELS.

K. Shahrokhi1, T. E. Cheatham3 and G. S. Yost1. 1Pharmacology & Toxicology, University of Utah, Salt Lake City, UT and 2Medicinal Chemistry and of Pharmacaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT.

The contribution of electronic, conformational and thermodynamic factors to P450-catalyzed reaction mechanisms should improve structural models. The lack of a consistent set of atomic parameters for the key heme species during the P450 catalytic cycle has limited the accuracy of structure based methods to predict metabolism and bioactivation of xenobiotics to DNA-binding reactive intermediates. We have used the P450-specific metabolism of tamoxifen—the most widely used drug for the treatment of breast cancer, as a molecular probe of competing metabolic pathways by several P450 enzymes to refine an integrated computational approach for predicting drug metabolism. Dehydrogenation of tamoxifen metabolites to electrophilic intermediates is one pathway that is linked to DNA adducts and carcinogenesis. Quantum mechanics calculations of key heme species during the P450 catalytic cycle at the UB3LYP/LACVP level of theory combined with molecular dynamics-refined P450 structures, and a novel quantum mechanics-based site-of-metabolism scoring scheme, illustrated the importance of modeling the drug–enzyme interactions during key steps of the P450 catalytic cycle. The improved model of CYP3A4 now predict for the first time, production of experimentally validated quinone methide intermediate of tamoxifen dehydrogenation. Supported by NIH grants # GM074249 from the National Institute of General Medical Sciences, NICHD Grant # HD060559 and # NCRR 1 S10 RR17214-01 from the National Center for Research Resources, NSF grant # MCA0178027 and the University of Utah Center for High Performance Computing.

**1097** CYP3A4 ACTIVE-SITE RESIDUES THAT MODULATE THE SELECTIVE DEHYDROGENATION OF RALOXIFENE TO ITS PROTEIN BINDING INTERMEDIATE.

C. Moore, K. Shahrokhi and G. S. Yost. Pharmacology & Toxicology, University of Utah, Salt Lake City, UT.

The use of molecular modeling in conjunction with site-directed mutagenesis has extensively been used to study substrate orientation with cytochrome P450 active-sites, and to identify potential residues involved in positioning and catalytic mechanisms of these substrates. However, because docking studies utilize static models to simulate dynamic P450 enzymes, the effectiveness of these studies are highly dependent on accurate enzyme models. We employed the cytochrome P450 3A4 (PDB code:1W0E) to predict the binding of metabolite of the known CYP3A4 substrate raloxifene using AutoDock 3.05. In addition, partial charges were incorporated into the P450 heme moiety. Dehydrogenation of raloxifene to its electrophilic di-quinone methide intermediate has been linked to the potent inactivation of CYP3A4. Based on our models, active-site residues involved in the positioning and/or catalysis of raloxifene supporting dehydrogenation were identified, and site-directed mutagenesis studies were conducted to validate our models. The addition of partial charges to the heme moiety dramatically increased accuracy of the docking studies, increased the number of conformations predicting dehydrogenation, and facilitated the identification of substrate/active-site interactions. Based on our improved model, we hypothesized that the F215 residue plays an important role in orienting raloxifene for dehydrogenation through a combination of electrostatic and steric interactions. Substitution of this residue with glycine or glutamine residues, F215G and F215Q respectively, significantly decreased dehydrogenation rates without concurrent changes in the rates of raloxifene oxygenation. Thus, the improved structural model predicted enzyme/substrate interactions that control the selective dehydrogenation of raloxifene to its protein-binding intermediate. Supported by NIGMS Grant GM074249 and NICHD Grant HD606559.

**1098** FLUTICASONE PROPIONATE, AN INHALED GLUCOCORTICOID, IS A POTENT INACTIVATOR OF CYP3A5, THE PREDOMINANT PULMONARY P450 ENZYME.

G. S. Yost, T. Murai, K. Shahrokhi, C. Moore and C. R. Orton. Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

Inhaled glucocorticoid (GC) therapy is a mainstay of asthma management. GCs are metabolized by members of the cytochrome P450 (CYP) 3A family in both liver and lung, but the enzymes are differentially expressed. Selective inhibition of one or more CYP3A enzymes could substantially modify target and systemic concentrations of GCs. The objective of the present study was to evaluate mechanism-based inactivation of three CYP3A enzymes by GCs and to identify reactive metabolites responsible for the inactivation process. The five major inhaled GCs, namely, beclomethasone dipropionate, budesonide, fluticasone (FLN), fluticasone propionate (FLP) and triamcinolone acetonide (TRI) were used. Reconstituent CYP3A4, 3A5 and 3A7 were used to evaluate mechanism-based inactivation and the formation of reactive metabolites. Reactive metabolites produced from each enzyme were trapped with GSH and analyzed using LC-MS and NMR. Quantum mechanics-based energy calculations were performed using Gaussian 03 software, to characterize the intermediates. FLT was the most potent mechanism-based GC inactivator of CYP3A5. It inactivated CYP3A5 in a time- and concentration-dependent manner with ta, partition ratio of 16 μM, 0.027 min- and 3.0, respectively. No CYP3A enzymes produced detectable GSH adducts of FLP; however, FLP and TRI, structural analogs of FLT, produced several GSH adducts and potentially reactive A metabolites. The energies of putative dehydrogenated intermediates indicated that A-FLT is an unstable high energy transition state intermediate species, whereas A-FLN is an energy minimized stable metabolite, which would not inacti-
The lateral nasal gland (LNG), an important organ for the detection of odorant signals and for the maintenance of the olfactory mucosa, is a target for toxic damage by a number of xenobiotoic compounds, including the drug acetaminophen (AP), in rodents. Previously, we found increased resistance to AP toxicity in the LNG of male Cyp2g1-null/Cyp2a3-low mice, compared to male wild-type (WT) mice (Zhang et al., 2011). Pharmacol. Exptl. Ther., 308, 719-728, 2004). The mechanisms underlying this increased resistance were explored in the present study. In the LNG of Cyp2g1-null/Cyp2a3-low males, compared to the LNG from WT males, we found lower tissue AP levels, lower extent of AP-induced depletion of non-protein thiol, lower microsomal activities toward testosterone (T), but no change in microsomal activities toward AP, as well as higher tissue levels of T and salivary androgen-binding proteins (ABPs). These findings, as well as subsequent studies on AP-protein adduct analysis, suggest the existence of a feedback defensive mechanism against AP-induced toxicity in the male LNG (and possibly in other secretory glands as well). In this mechanism, excessive exposure to xenobiotoic compounds leads to inhibition of P450 enzymes involved in T metabolism, consequent increases in tissue T levels, and up-regulation of secretory carrier proteins (e.g., ABPs), which are capable of protecting the tissue against xenobiotoics and/or their reactive metabolites through the formation of protein adducts. This mechanism explains why decreases in CYP2A5 expression can lead to increased resistance of the LNG to AP toxicity, and provides a novel link between androgen homeostasis and tissue resistance to chemical toxicity. (Supported in part by NIH grant ES-007462)

1099 A NOVEL FEEDBACK DEFENSIVE MECHANISM AGAINST ACETAMINOPHEN-INDUCED TOXICITY IN THE LATERAL NASAL GLAND: INVOLVEMENTS OF CYP2A5-MEDIATED ANDROGEN METABOLISM AND MODULATION OF THE PRODUCTION OF SALIVARY ANDROGEN-BINDING PROTEINS.

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The long-term goal of this project is to assess potential harmful effects of poultry and swine feed additives, Roxarsone and Arsalanate. Current farming practices, especially the Concentrated Animal Feed Operations (CAFOs) and recycling of poultry litter to contaminate farmland, may increase exposure to these contaminants, which may increase exponentially since the 1980s as CAFOs increased in number. Although efforts are underway to change this practice, leaching into ground water will likely cause continuing contamination. Roxarsone and the metabolites AHB, acetarsone and Arsalanate and its major metabolite Arsanetin (4-N-acetylasarancil) have been studied. Our work reveals that: 1) In Caco-2 cells which model human intestinal cells, all five compounds cause increased proliferation; 2) AHB, the N-reduced metabolite of Roxarsone, is the most proliferative, as measured by 3H-thymidine uptake and confirmed by flow cytometry; 3) Acetylation, glucuronidation, and sulfation reaction rates are poor with AHB, indicating that bioactivation by CYP450 is the major pathway. Increased cell proliferation was associated with increased AR expression and AR-mediated transcriptional activity. (Supported in part by NIH grants AG026329 and RCMI RR03032.)

1100 ROLES OF CYTOCHROME P450 REDUCTASE IN MODULATING AMYLOID BETA PLATEAUX FORMATION IN A MOUSE MODEL OF ALZHEIMER’S DISEASE.

J. Guo, C. Fang, Y. Yao, H. Luo, V. Bolivar, W. Yang and X. Ding, Wadsworth Center, Albany, NY.

The expression and function of cytochrome P450 reductase (CPR), the obligate electron donor for all microsomal P450 enzymes, can be modulated by exposures to certain xenobiotoic compounds, which may either induce CPR expression or inhibit CPR activity. The aim of the present study is to test the hypothesis that CPR/P450 enzymes play a role in the etiology of Alzheimer’s disease (AD), either through CPR/P450-mediated production of reactive oxygen species, or through CPR/P450-mediated metabolism of endogenous regulatory molecules (e.g. cholesterol and sex steroids) that can influence amyloid β (Ab) formation and neuron degeneration in AD. To test this hypothesis, we utilized a recently developed transgenic mouse model (named Cpr-low), in which there are >70% decreases in the levels of CPR expression in all organs examined, including the brain. The Cpr-low mice were crossed with amyloid precursor protein (APP)-transgenic mice (Tg2576); the resultant APP/Cpr-low double transgenic mice were studied, in order to determine the impact of the CPR loss on APP-associated pathological and functional changes. A significant reduction in the levels of Ab peptides (at 12 months of age) and abundance of Ab plaques (at 18-21 months of age) was observed in the APP/Cpr-low mice, accompanied by a marked alleviation of contextual memory deficit that was seen in the control APP mice (measured by fear conditioning tests at 12-14 months of age). Our findings provide the first in vivo evidence for the role of CPR and CPR-dependent enzymes in modulating Ab plaque formation in a mouse model of AD. Future studies on the ability of xenobiotoic inducers or inhibitors of CPR to modulate Ab plaque formation are warranted. (Supported in part by NIH grant AG026329).

1101 CACO-2 CELL PERMEATION OF FIVE BENZARSONATES INCREASES THE LIKELIHOOD OF HEPATIC BIOTRANSFORMATIONS. D. K. Robinson1, K. Jackson1, L. Hammonds-Odie1, K. Ward1 and G. S. Bayes1, 1Chemistry, Spelman College, Atlanta, GA, 2Pharmacology and Toxicology, Morehouse School of Medicine, Atlanta, GA and 3School of Science and Technology, Georgia Gwinnett College, Lawrenceville, GA.

The expression and function of cytochrome P450 reductase (CPR), the obligate electron donor for all microsomal P450 enzymes, can be modulated by exposures to certain xenobiotoic compounds, which may either induce CPR expression or inhibit CPR activity. The aim of the present study is to test the hypothesis that CPR/P450 enzymes play a role in the etiology of Alzheimer’s disease (AD), either through CPR/P450-mediated production of reactive oxygen species, or through CPR/P450-mediated metabolism of endogenous regulatory molecules (e.g. cholesterol and sex steroids) that can influence amyloid β (Ab) formation and neuron degeneration in AD. To test this hypothesis, we utilized a recently developed transgenic mouse model (named Cpr-low), in which there are >70% decreases in the levels of CPR expression in all organs examined, including the brain. The Cpr-low mice were crossed with amyloid precursor protein (APP)-transgenic mice (Tg2576); the resultant APP/Cpr-low double transgenic mice were studied, in order to determine the impact of the CPR loss on APP-associated pathological and functional changes. A significant reduction in the levels of Ab peptides (at 12 months of age) and abundance of Ab plaques (at 18-21 months of age) was observed in the APP/Cpr-low mice, accompanied by a marked alleviation of contextual memory deficit that was seen in the control APP mice (measured by fear conditioning tests at 12-14 months of age). Our findings provide the first in vivo evidence for the role of CPR and CPR-dependent enzymes in modulating Ab plaque formation in a mouse model of AD. Future studies on the ability of xenobiotoic inducers or inhibitors of CPR to modulate Ab plaque formation are warranted. (Supported in part by NIH grant AG026329).
gene are associated with diseases such as cancer. Very little is known about the in vivo regulation of mEH. The purpose of this study was to determine the tissue distribution, ontogeny, and chemical induction of mEH in mice. For tissue distribution, thirteen tissues were collected from 8-week-old wild-type mice. For ontogeny, livers were collected from two days before birth through various postnatal ages. For chemical induction, male mice were administered microsomal enzyme inducers that activate critical transcription factors regulating xenobiotic metabolism, including AhR (TCDD), CAR (TCPOBOP), PXR (PCN), PPARα (clofibrate), and Nrf2 (oleylacetate). Total RNA was isolated, and mEH mRNA expression was determined by the qDNA assay. The mEH mRNA was highest in liver and testis in wild-type mice. The mRNA expression of mEH was low in prenatals, but increased with age in both males and females. The mEH mRNA was not readily induced by activators for AhR, PXR, or PPARα. In contrast, both CAR and Nf2 activators induced mEH mRNA in liver. This induction was blocked in CAR- and Nf2-null mice, indicating the induction was CAR- and Nf2-dependent. In silico analysis identified putative binding sites for CAR and Nf2 in and around the mEH gene locus. In conclusion, the mEH mRNA was found to be enriched in liver and testis, its expression in liver gradually increased with age, and mEH expression in liver can be induced through CAR and Nf2 activation. (Supported by NIH grants ES01714, ES009649, ES009716, ES015714, RR021940)

**1104 TRANSCRIPTIONAL AND TISSUE SPECIFIC REGULATION OF HUMAN MICROSOMAL EPOXIDE HYDROLASE (EPHX1) EXPRESSION IS MODULATED BY DIETARY ISOTHIOCYANATE DERIVATIVES.**

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Microsomal epoxide hydrolase (EPHX1) is a critical metabolic enzyme that catalyzes the hydrolysis of electrophilic epoxides to less reactive dihydrodiols, thereby playing an important role in the detoxification of ROS and xenobiotic epoxide intermediates. However, under some circumstances, the enzyme bioactivates substrates, in particular the carcinogenic polycyclic aromatic hydrocarbons present in cigarette smoke. Our studies have determined that there are two prominent EPHX1 transcriptional variants present in human tissues. The transcripts are generated from the use of alternative gene promoters that produce products containing different domains, referred to as E1 and E1b, respectively. The E1-containing transcript is expressed most exclusively in liver while the E1b transcript, driven by the use of a promoter residing 18.5 kb upstream from the coding region of the gene, is the predominant EPHX1 transcript generated in all tissues. The regulatory mechanisms directing the respective expression levels and tissue-specific patterning of the E1 and E1b transcripts is an area of great interest. In this study, we investigated the effects of dietary isothiocyanate derivatives as potential modulators of EPHX1 expression. These compounds are under intensive study as potential chemopreventive agents in human trials. We determined that sulforaphane as well as 3-phenylpropyl isothiocyanate can potently and differentially regulate EPHX1 transcription in liver and lung tissue. The pattern of expression for the E1 and E1b transcripts was confirmed with protein immunoblotting experiments. These data suggest that the molecular mechanism of human EPHX1 regulation includes antioxidant response signaling, likely mediated through the nuclear factor erythroid 2-related factor 2 (Nrf2) master regulator pathway.

**1105 OXIDATION OF 4-CHLOROBIPHENYL METABOLITES TO THEIR FREE RADICAL SPECIES BY PROSTAGLANDIN H SYNTHASE-2.**

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Polychlorinated biphenyls (PCBs) are a class of environmental pollutants that have entered the environment through both their use and disposal. PCBs are mainly metabolized by hepatic cytochrome P450 (CYPs) to mono-, and dihydroxyl metabolites, which can undergo redox interconversion to quinones (Q). In our previous study we found that prostaglandin H synthase-2 (PGHS-2), an inducible enzyme found basally in extra-hepatic tissues, catalyzes the oxidation of dihydroxy PCB metabolites to corresponding Q as identified by LC-MS. Here we show that PGHS-2 also plays an important role in catalyzing the oxidation of these PCB metabolites to semiquinones (SQd-). Using 4-chlorobiphenyl-2,3'-5'-hydroquinone (4-CB-H2Q) as a model compound, two SQd- species were detected by electron paramagnetic resonance (EPR) spectroscopy. These results indicate that PGHS-2 is capable of catalyzing the oxidation of lower chlorinated PCB hydroquinones to Q by two sequential one-electron oxidations generating SQd-as intermediates. This study demonstrates the formation of SQd- and Q from PCB-metabolites by PGHS-2, and underscores the potential role of PGHS-2 in the metabolic activation of PCBs in extra-hepatic tissues. (Supported by NIH P42 ES 013661 and ES 560505.)

**1106 MICRONUCLEUS FREQUENCIES AND DNA DAMAGE IN MALE MICE ADMINISTERED HYDROXYUREA.**

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Hydroxyurea (HU), a chemotherapeutic agent, is the only effective medication for treatment of sickle cell disease in adults. Insufficient information about its long-term toxicity in young children—a growing patient population—remains a major concern. To evaluate the subacute genotoxic potential of HU in a rodent model, the NTP conducted two studies in male B6CsF1 mice using a combined micronucleus (MN)/Comet assay. HU (33-500 mg/kg) was administered by gavage once daily for 4 consecutive days. The highest dose was also dosed on the basis of the maximum low toxicity (restriction in % reticulocytes (RET)) in a range finding study and the lowest doses were selected to bracket the human equivalent dose. Blood samples were collected 4 hr after the final dosing for measurement of MN-RET frequencies by flow cytometry and samples of liver, blood, stomach, and colon were collected and prepared for assessment of DNA damage using the pH-13 Comet assay. A significant, dose-related increase in MN-RET (p < 0.0001) was observed in the mice administered HU; considerable bone marrow toxicity, assessed by a decrease in the %RET, was also seen in the mice receiving doses ≥ 125 mg/kg. An observed downward shift in the MN median channel fluorescence intensity suggests the likelihood of highly fragmented DNA within the MN. DNA damage measured in the Comet assay was evident in cells from the stomach and liver of HU-treated mice at all doses tested, but no damage was detected in blood or colon cells. The observed damage appeared to correlate with significant increases in low molecular weight DNA, indicative of cytoxicity (apoptosis or necrosis), and/or lesion sizes. The lowest doses of HU tested. Together, the data obtained from this combined MN/Comet assay demonstrate significant genetic damage and associated cytotoxicity in B6CsF1 mice resulting from HU treatment. Supported by NIEHS/NTP contract N01-ES-35514.

**1107 EVALUATION OF PHOTOGENOTOXICITY IN THE IN VITRO MICRONUCLEUS ASSAY IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES.**


Recent demonstration of pseudophotoclastogenicity has cast doubt on the specificity and utility of current test systems used for detecting photogenotoxicity. However, studies to date largely have been performed in transformed cell lines that are genetically unstable. Thus, we adapted the in vitro micronucleus (MN) assay in human peripheral blood lymphocytes (HPBL) to evaluate photogenotoxicity. Venous blood was collected and HPBL were isolated and stimulated to grow (t = 0) in mass culture. Individual 5 mL cultures were prepared in 60-mm dishes (t = 48 hr) and treated with 8-methoxypsoralen (8-MOP) or the concurrent vehicle and unirradiated positive controls. Cultures were pre-incubated with the test and control concentrations for the dark for ~10 minutes, and half of the cultures were irradiated with a Xenon arc solar simulator lamp with a UV filter (290 nm cut off). Cultures were re-incubated until the end of treatment (t = 51 hr), washed by centrifugation, and transferred to media containing cytochalasin B. Cultures were harvested (t = 72 hr) and slides were prepared and stained with Giemsa and May-Grunwald. For each culture, 200 cells were scored for cytotoxicity (cytochalasin B blocked proliferation index, CBPI), and 400 binucleated cells were scored for micronuclei (%MN-BN). Statistically significant, dose-dependent increases in %MN-BN were induced in the vehicle control cultures by extended UV exposure (as compared to unirradiated controls), as well as in cultures treated with 8-MOP that were irradiated for at least 50 seconds (as compared to the matched irradiated controls). In contrast, 8-MOP was uniformly negative in the absence of light exposure. These results demonstrate the feasibility of detecting micronuclei induced by photogenotoxic compounds in HPBL. Additional validation studies with lomeloxacin and nalidixic acid are ongoing.
**STYRENE-INDUCED TOXIC EFFECTS IN ALDH2 KNOCKOUT MICE.**

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Styrene is an important chemical widely used in manufacturing plastics and synthetic rubber. Styrene exposure has been reported to cause DNA damage as shown by the increased DNA adducts and chromosomal aberrations. This effect has been correlated with the genetic polymorphisms of metabolizing enzymes such as CYPs and GSTs, and DNA repair enzymes. Aldehyde dehydrogenase 2 (ALDH2) is also involved in the metabolism of styrene, but the enzyme activity is deficient in about 40 percent of East Asian population due to a mutant allele of the gene encoding the enzyme. In this study, we used ALDH2 gene knockout (KO) mice to detect any difference in styrene induced effects such as micronuclei and other endpoints compared with those in wild type (WT) mice. Male and female mice were administrated with styrene at 0, 100, 400 and 800 mg/kg, p.o., per day, 5 days per week, for 4 consecutive weeks. Peripheral blood was sampled 24 hr after the last treatment and the micronucleus in the reticulocytes was detected by a flow cytometric method using kit from Litron Laboratories. The background of the frequency of micronucleated reticulocytes (MN-REts) was higher in KO mice than in the WT mice. The control and test article groups were treated with the vehicle, corn oil, or cyclophosphamide (CPA) at 40 mg/kg as the positive control. The control and test article were both orally administered to induce micronuclei in polychromatic erythrocytes (PCEs) in the bone marrow of the mouse to evaluate clastogenic potential. In the dose range-finding phase, 2000 mg/kg body weight was determined suitable as it was the maximum guideline-recommended dose. In the definitive study, 1-menthone was then administered orally at dose levels of 500, 1000 or 2000 mg/kg in corn oil to NMRI mice (5/sex/dose). The control groups were treated with the vehicle, corn oil, or cyclophosphamide (CPA) at 40 mg/kg as the positive control. The control and test article were both orally administered at a volume of 10 ml/kg body weight. Bone marrow cells were harvested 24 and 48 h after the treatment and examined microscopically for the presence of micronuclei. At least 2000 PCEs per animal were scored for micronuclei. There was no statistically significant increase in the incidence of micronuclei in either male or female mice and is not genotoxic.

**EVALUATION OF L-MENTHONE IN AN IN VIVO MOUSE MICRONUCLEUS TEST.**

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A mouse micronucleus test was conducted on 1-menthene (CAS # 14073-97-3), a widely used fragrance ingredient, to determine its potential to induce micronuclei in the polychromatic erythrocytes (PCEs) in the bone marrow of the mouse to evaluate clastogenic potential. In the dose range-finding phase, 2000 mg/kg body weight was determined suitable as it was the maximum guideline-recommended dose. In the definitive study, 1-menthene was then administered orally at dose levels of 500, 1000 or 2000 mg/kg in corn oil to NMRI mice (5/sex/dose). The control groups were treated with the vehicle, corn oil, or cyclophosphamide (CPA) at 40 mg/kg as the positive control. The control and test article were both orally administered at a volume of 10 ml/kg body weight. Bone marrow cells were harvested 24 and 48 h after the treatment and examined microscopically for the presence of micronuclei. The number of PCEs showed no statistically significant increases when compared with the control. Again this toxic effect was only found in the KO, but not WT mice. These results suggest that ALDH2 polymorphism is another genetic factor that may modify the toxic effects of styrene and may be used as a biomarker of susceptibility to styrene exposure.

**EVALUATION OF ISOAMYL ALCOHOL IN AN IN VIVO MOUSE MICRONUCLEUS TEST.**

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Isoamyl alcohol, a widely used fragrance ingredient, was tested in an in vivo mouse micronucleus assay to evaluate the clastogenic potential as measured by its ability to induce micronucleated polychromatic erythrocytes in mouse bone marrow. This test is a short-term in vivo cytogenetic assay for detecting agents that induce chromosomal breakage and spindle malfunction. In the dose range-finding phase, 2000 mg/kg body weight was determined suitable as it was the maximum guideline-recommended dose. The definitive micronucleus study consisted of several groups, each containing 5 male and 5 female NMRI mice. The control groups were treated with the vehicle, corn oil, or cyclophosphamide (CPA) at 40 mg/kg as the positive control. Isoamyl alcohol was tested at dosages of 500, 1000, or 2000 mg/kg. Both the test and control articles were administered via gavage at a dose volume of 10 ml/kg body weight. Bone marrow cells (polychromatic erythrocytes, PCEs) were collected 24 and 48 h after the treatment and examined microscopically for the presence of micronuclei. At least 2000 PCEs per animal were scored for micronuclei. There was no statistically significant increase in the incidence of micronuclei at doses exceeding 2000 mg/kg. The 2000 mg/kg dose showed a trend towards significant increase in micronuclei when compared with the control. Again this toxic effect was only found in the KO, but not WT mice.

**DOSE-RESPONSE CHARACTERIZATION OF VINYL ACETATE AND ACETALDEHYDE-INDUCED MICRONUCLEI IN HUMAN TK6 CELLS.**

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An in vivo mammalian erythrocyte micronucleus test was conducted to evaluate the potential of gamma-nonalactone (CAS No. 104-61-0), a widely used fragrance material, to induce micronuclei in polychromatic erythrocytes (PCEs) in the bone marrow of the mouse. The study was conducted in compliance with GLP and OECD Test Guideline 474 (Mammalian Erythrocyte Micronucleus Test). Male and female NMRI mice (5/sex/dose) were administered gamma-nonalactone orally at dose levels of 500, 1000 or 2000 mg/kg body weight in corn oil at a dose volume of 10 ml/kg body weight and sacrificed at 24 or 48 hours after dosing. Control groups received the vehicle alone or cyclophosphamide (40 mg/kg body weight) as a positive control. PCEs were collected at 24 and 48 hours after administration and examined microscopically for the presence of micronuclei. At least 2000 PCEs per animal were scored for micronuclei. The number of PCEs was not substantially decreased in gamma-nonalactone treated groups compared to the mean value of PCEs of the vehicle control group indicating that gamma-nonalactone did not produce any cytotoxic effects in the bone marrow. There was no significant increase in the incidence of micronucleated PCEs in gamma-nonalactone treated groups relative to their respective vehicle controls in either male or female mice, regardless of dose level or bone marrow collection time. The mean values of micronuclei observed in the gamma-nonalactone treated groups were below or near vehicle control values. Cyclophosphamide induced a statistically significant increase in micronucleated PCEs in both male and female mice. It was concluded that under the conditions of the test, gamma-nonalactone was not genotoxic.
CHO cells were seeded in 96-well microtiter wells. Mannitol (negative control), Mitomycin C (clastogenic control), Colchicine, Etoposide and Staurosporine were used in increasing concentrations. Baseline wells were fixed at the time of compound introduction. Each chemical was evaluated with and without Cytochalasin B (CB). After 24 hour incubations, plates were stained with DAPI and FITC and analyzed on the iCys ® Laser Scanning Imaging Cytometer (Compucyte, Westwood, MA). A comprehensive data analysis protocol enabled cellular segmentation based on cytoplasm staining (FITC); nuclei/MN segmentation was based on DNA/DAPI staining. Data without DAPI staining. Reported parameters included cell counts, DNA content per cell. MN frequency, proliferative index defined as the ratio of the cell counts of the test wells to the baseline values. Results: In experiments without cytochalasin B, negative controls were characterized by increased proliferation and an exponential cell cycle distribution. Positive controls (Mitomycin C and experimental compounds) induced expected dose-response increases in micronuclei frequency, dose dependant decreases of the proliferative index and corresponding changes to the cell cycle distributions. Conclusions: 1. MN assay by automated lasers imaging scanning cytometry can accurately and reproducibly detect micronuclei induction by clastogens and aneugens. 2. Toxicological effects of compounds can be evaluated simultaneously with MN. 3. Cytochalasin B method provides better sensitivity in clastogenic compounds, but ambiguous results with aneugens. Parallel analysis by both methods is recommended. 4. Compucyte’s laser scanning cytometry platform offers an additional advantage of accurate and precise DNA content / cell cycle measurements in addition to standard MN counts without requiring additional markers.

**1115 AUTOMATED IN VITRO MICRONUCLEUS SCORING REPRESENTS AN EFFICIENT TOOL FOR EVALUATING CHEMICALS’ GENOTOXIC THRESHOLDS.**


It has been argued that even DNA-reactive genotoxicants can exhibit thresholds below which no genotoxic damage occurs. This lab has developed an automated approach for scoring in vitro micronuclei (In Vitro MicroFlow®) that may be useful for investigating this hypothesis. This method provides a suspension of free nuclei and micronuclei (MN) that are compatible with flow cytometric analysis. Experiments described herein were performed to evaluate the ability of this method to characterize the dose-response relationship that exists at low concentrations of several genotoxicants. Initial experiments were conducted using two simple models to establish whether or not the analytical system was able to detect linear dose-response relationships. In one model, mixtures of latex beads were used to simulate a range of MN frequencies, and in a second model, TK6 cells with elevated MN frequency were added in varying proportions to untreated cells at the same cell density. Statistical modeling indicated that the data are best described as having a linear fit, and they also suggested that analyses benefited from scoring MN frequency in 100,000 cells per replicate, although this was only marginally superior to a stop mode of 20,000 cells. Subsequently, TK6 cells were used to study each of 6 prototypical genotoxicants: colchicine, vinblastine sulfate, EMS, MMS, ENU, and MNU. Treatments occurred for 24 – 30 hrs, and were performed in 4 replicate wells per concentration. For each compound, the so-called hockey stick model of Luiz & Luiz [Mutat. Res., 118 (2009) 118-122] gave a significantly better fit of the data compared to a linear model. These results suggest that flow cytometry represents a practical approach for studying the low concentration portion of the dose-response curve, and lends support to the hypothesis that non-linear models will often describe the dose-response relationship of genotoxicants, even for DNA-reactive clastogens.
1118 THE PRESENCE OF DIETHYL FUMARATE IMPACTS
CLASTOGENICITY TEST RESULTS FOR DIETHYL 2-
ETHOXYSUCCINATE IN CHINESE HAMSTER OVARY
CELLS.

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Diethyl 2-ethoxysuccinate (DETS) is an aliphatic diester for which little toxicity
information is available; it is structurally similar to many compounds that are
naturally occurring in food, or used as food flavoring agents. When evaluated
using Structure Activity Relationship (SAR), DETS was not found to possess struc-
tural alerts to suggest potential for genotoxicity. However, initial results from an in
vitro chromosomal aberration assay using Chinese Hamster Ovary cells (CHO)
demonstrated that 20 hour DETS treatment at 500 μg/ml and 1000 μg/ml, with-
out metabolic activation, produced a clastogenicity rate of 11% and 52%, respec-
tively relative to the DMSO control group. Increased rates of clastogenicity were
not seen following 4 hour treatment with or without metabolic activation.

Although the DETS sample purity that might reduce the initial assay was identified as 98.5%,
it was considered likely that a contaminant was producing such a high rate of clas-
togenicity. Further evaluation showed that the original sample contained less than 1.3% diethyl fumarate, which has previously been positive for clastogenicity in an
in vitro chromosomal aberration assay. When the DETS sample was further puri-
ﬁed (>99.9% purity) and the diethyl fumarate removed, increased rates of clasts
were not seen in a repeat of the 20 hour treatment without metabolic acti-
vation. Further evidence for the lack of genotoxicity of DETS was found when
DETS was negative in an Ames mutagenicity assay. These results demonstrate the
importance of complete characterization of test samples and the potential
for significant impact of low level impurities, especially in highly sensitive in vitro
assays for genotoxicity.

1119 EVALUATION OF ZIDOVUDINE (AZT)-INDUCED
GENOTOXICITY CHANGES IN HUMAN CELLS
EXPOSED TO THE CYTOPROTECTIVE AGENT
WR1065, USING THE CYTOASSAY.

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AZT, used for therapy of HIV-1, becomes incorporated into the DNA of virus and
host cells inducing a variety of genotoxic changes. Because of this it is important to
search for the AZT that might reduce in the initial assay was identified as 98.5%;
radioprotective agent WR1065 reduces AZT-induced mutagenesis, and here we ex-
plor the cytoprotective potential of this compound in MOLT-3 human lymph-
phoblatoïd cells exposed to plasma levels of AZT. Cells were grown for 2 passages
(approximately 6 days) in 0 or 10μM AZT and then subcultured into 12- wells plate,
in 20μl of culture medium. After 24 hrs, clastogenic rates of clastogenicity were not
seen in a repeat of the 20 hour treatment without metabolic acti-
vation. Further evidence for the lack of genotoxicity of DETS was found when
DETS was negative in an Ames mutagenicity assay. These results demonstrate the
critical importance of complete characterization of test samples and the potential
for significant impact of low level impurities, especially in highly sensitive in vitro
assays for genotoxicity.

1117 INVESTIGATION OF THE GENOTOXIC EFFECTS
OF THREE BLACK TONER POWDERS IN CULTURED
HUMAN EPITHELIAL A549 LUNG CELLS.

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4Department of Environmental Science, University of Fribourg, Fribourg, Switzerland.

Previously, adverse effects from inhalation of toner powders or from emissions dur-
ing laser printing were considered minimal. However, recent reports suggest possi-
ble significant adverse health effects from toner dust inhalation. The aim of the study
was to evaluate the cytoxic and genotoxic potential of three commercially
available toner powders in vitro. Human lung carcinoma epithelial A549 cells were
exposed to toner-powder suspensions and subjected to different bioassays, i.e.
LDH, COMET and micronucleus assay. Toner powders were physically/chemically
examined by SEM, EDX and AAS. PAH content in the toner powders was deter-
mined by hot extraction (toulen 100°C) and GC-MS. The investigated toner powders
consist of C-bearing, rounded to slightly elongated particles with diameters
of 2 to 8 μm. The particle surface is somewhat rough and is covered by rounded
(nano)particles (30-200nm). The toner powders contain relatively high amounts of the heavy metals Fe (to 28000 ppm), As (to 31 ppm), Ni (to 24 ppm), Pb (to 1 ppm)
and Zn (to 73 ppm). Concentration of 35 PAHs in the three toner powders ranged from 404 ppb up to 2623 ppb. All three toner powders induced LDH leak-
age, DNA damage and formed micronuclei, albeit to a varying extent. Overall, the
study suggests that the investigated toner powders are both cytoxic and genotoxic
in human lung cells in vitro. Compared to the positive particle control quartz
DQ12, the magnitude of the toxicity of the toner powders was less to equal. From the
physical/chemical analysis it can be concluded that the heavy met-
als are responsible for cytoxic effects, whereas the PAHs induce genotoxic effects.
Which specific compounds are responsible for the toxic effects observed will be the
focus of further studies.

1120 ASSESSMENT OF THE IN VIVO CYTOGENETIC
POSSIBILITY OF PETROLEUM-DERIVED SUBSTANCES.

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The petroleum industry has assessed the clastogenic potential of petroleum derived
materials in cytogenetic assays (e.g., chromosome damage or micronucleus forma-
tion in bone marrow cells). Early studies suggested that petroleum substances did
not produce clastogenic effects under in vivo conditions regardless of substance
tested or test method utilized. This was surprising as some aromatic hydrocarbons,
e.g., benzen, benzo(a)pyrene, dimethyl benzanthracene, have produced cyto-
genetic damage when tested under similar conditions. Recently a new data set em-
ploying the micronucleus test was compiled consisting of 20 petroleum substances
which contained varying levels of polycyclic aromatic constituents. All substances
were tested using a common protocol involving repeated dermal administration to
rats. Of the 20 substances tested, 18 were reported as negative and 2 positive. One
of the two positive outcomes was not dose-related and results contradicted earlier
negative chromosome aberration and micronucleus studies with the same material
and in the other case the small but statistically significant increase seen in female
rats was not replicated in males. Accordingly, it was concluded that these 2 positive
outcomes were most likely to have been spurious findings. Despite the absence of
Chinese hamster lung fibroblasts (V79) for: (a) inhibition (stabilization) of TOPO II
using the Topogen in vivo link kit (TOPOGEN); (b) induction of phosphory-
lation of histone H2AX (γH2AX), a response to DNA double-strand breaks
(DSBs) assessed by immunohistochemistry and flow cytometry; and (c) induction of
DSBs using the neutral single cell gel electrophoresis (comet) assay. The FQs
Clinafloxacin (CLINA), Gatifloxacin (GATTI) and Lomefloxacin (LOME), were
studied over a dose range and at a specific effective equimolar concentration (0.175
mM). The positive control comparator was Etoxfed (ETO), a TOPO II inhibitor.
V79 cells were exposed to the test substances in producing inhibition of TOPO II,
induction of γH2AX and DSBs. Comparatively, ETO was about 300 times more potent
than the FQs in inhibiting inhibition of TOPO II. At the comparator concentrations,
CLINA was the most potent followed by GATI and LOME. In the γH2AX and comet assays,
a similar rank order was evident. Thus, the genotoxicities of these FQs are consistent
with inhibition of TOPO II.
clastogenic effects, a number of samples did affect hematological parameters, pro-
viding empirical evidence of target tissue involvement. More limited studies using in
vitro protocols produced similar results. These in vitro results suggest that the
negative results in vivo are not due to a lack of genotoxic potential, but an indication that the substances being tested lack in vivo clastogenic potential. It was concluded that petroleum-derived materials are unlikely to produce clastogenic ef-
facts in bone marrow assays regardless of test or sample conditions.

DNA DAMAGING AND CLASTOGENIC EFFECTS OF PHYTOCHEMICALS PARTIALLY ISOLATED FROM CRUDE EXTRACT OF GLINUS LOTOIDES.

A methanolic extract of Glinus lotoides, a medicinal plant used in Africa and Asia
for various therapeutic purposes, was recently shown to cause DNA damage in vitro. To further explore the potential genotoxicity of this plant, fractionation of the crude extract was performed using reverse phase-depletion and a stepwise gra-
dient elution of methanol in water. Four fractions were collected and subsequently
analyzed for their DNA damaging and cytoxic effects in mouse lymphoma cells using an alcaline version of the comet assay and a cytokinesis-blocked version of the microcolony assay. To identify the presence of the genotoxicity non-genotoxic
principles, each fraction was subjected to LC-MS and LC-MS/MS analyses, fol-
lowed by database and literature research. While fractions containing flavonoids and oleane-type saponins or their mixture produced neither DNA nor chromosomal
damage, those containing hopane-type saponins or their mixture produced neither DNA nor chromosomal

DISCRIMINATION OF CLASTOGENIC AND ANEUGENIC COMPOUNDS IN HUMAN LYMPHOCYTES BY IMMUNOFLUORESCENT TECHNIQUES IN THE CB MICRONUCLEUS TEST.

The micronucleus assay in human lymphocytes was developed as a short term
testing system for the detection of both clastogenic and aneugenic chemicals. For human lymphocytes it is recommended to score micronuclei by the cytokinesis
block (CB) method using cytochalasin B. The original method developed by Fenech and Morley, 1985, focuses exclusively on binucleated cells. However, re-
cent studies suggest that micronuclei in mononucleated cells could provide comple-
mentary information. Results obtained with aneugenic compounds show a dose-
dependent increase of micronuclei in mononucleated cells. At present, the underlying mechanism has not been clearly indentified. In order to obtain more in-
famous immunefluorescence techniques were employed including CREST
analysis for detection of kinocentre proteins and staining of phosphorylated histo-
tone H2AX (γH2AX). The CREST analysis reveals whether micronuclei in mononucleated cells contain chromosomal fragments or whole chromosomes. The γH2AX staining detects phosphorylation of histone H2AX at serine 139 rapidly oc-
curring at sites flanking DNA double strand breaks. Our results suggest that mi-
cronuclei in mononucleated cells can be used to investigate the aneugenic activity of chemicals in a fast and easy way, and can be included in the CB assay with
human lymphocytes.

GADD45 INDUCTION IN THE GREENSCREEN HC INDICATOR ASSAY DOES NOT OCCUR INDEPENDENTLY OF CYTOTOXICITY.

Mammalian chromosomal integrity assays are influenced by cytotoxicity, a phe-
nomenon which impacts data interpretation, assay specificity and regulatory testing
guidelines. Concordance of the GADD45st GreenScreen HC indicator assay to es-
tablished in vitro and in vivo genetic toxicology assays has previously been de-
scribed, yet a detailed description in the manner by which cytotoxicity influences its performance has not. Here we present a post-hoc analysis of a previously tested set of 91 proprietary and non-proprietary compounds investigating the interaction between GADD45st induction and cytotoxicity as well how varying assay threshold
criteria influences concordance. GADD45st induction strongly correlates with cy-
totoxicity for the majority (72%) of compounds causing a positive GADD45st re-
response. Furthermore, modification of the GADD45st induction and cytotoxicity
test was not produced in any assay (framed to 68%) and concordance (from 55 to 68%), though a concomitant decrease in specificity is observed (from 97 to 68%). Additionally, an analysis of Roche proprietary
compounds tested in the micronucleus test demonstrates micronucleus induction is also influenced by cytotoxicity, albeit in an attenuated manner if compared to the GreenScreen HC indicator assay. We conclude that GADD45st induction in the GreenScreen HC indicator assay is influenced by cytotoxicity and that assay per-
fomance can be improved if different assay criteria are implemented.

CHEMICAL EXPOSURE AND THE GENERATION OF COPY NUMBER VARIANTS (CNVs).

Until recently, single nucleotide polymorphisms (SNPs) were thought to be the pre-
dominant form of genomic variation and to account for much of the normal phe-
o typic variation. Recent developments and applications of genome-wide tech-
ologies have lead to the discovery of thousands of copy number variants (CNVs: defi
ned as a gain or loss of DNA sequence measuring 1 kilobase and larger in size) in
the human genome. Human genomic copy number variation has been studied for
a number of years, but it was assumed that CNVs were few in number, had a rela-
tively limited impact on the total amount of genetic variation, and were mainly
associated with highly penetrant disease phenotypes. CNVs that did not result in
early-onset, highly penetrant genomic disorders were presumed to be neutral in
function, but the role of CNVs in complex diseases is now becoming increasingly
more appreciated. At this time, the factors and mechanisms that generate sponta-
neous CNVs are not well defined, including the potential role of exposure to chem-
ical stressors. To investigate the role of chemical exposure in the generation of
CNVs, an assay utilizing the zebrafish model system was developed. Use of the ze-
brafish model system in this assay presents many strengths including the fact that
CNVs, similar to the human genome, is also copy number variable. The zebrafish
model system in this assay presents many strengths including the fact that

zebrafish cells were first exposed to the known genotoxic chemical ethyl methanesulfonate (EMS). EMS exposure did result in the generation of spontaneous CNVs and interestingly CNVs were detected in similar genomic regions among multiple exposure concentrations. This study is confirming that chemical exposure can generate spontaneous CNVs and provide the basis for future studies aimed at investigating the potential for environmental chemical contaminants to generate spontaneous CNVs, the mechanisms by which these spontaneous CNVs are generated, and the biological significance of these alterations.

Bowhead (Balaena mysticetus) lung cells are resistant to chromium-induced damage. Chromium is a common environmental pollutant and a known human carcinogen with a genotoxic mechanism. We compared chromium genotoxicity in bowhead whale and human cells. Bowhead cells were more resistant to chromium-induced cytotoxicity and genotoxicity than human lung cells. For example, concentrations of 0, 0.5, and 1 μg/cm² lead chromate induced damage in 2, 10, and 8 percent of metaphases in bowhead cells, respectively; and 5, 34, and 42 percent of metaphases in human cells. The differences in effects were not due to individual animal differences as cells from a different bowhead whale showed the same trend. However, when we measured genotoxicity on a finer scale, we found no significant differences. For example, using gamma-H2AX foci formation as a measure, concentrations of 0, 0.5, and 1 μg/cm² lead chromate induced 5.1, 5.9, and 10.5 average foci per cell, respectively, in bowhead cells; and 3.7, 3.7, and 9.5 average foci per cell, respectively, in human cells. It is unclear why the different genotoxicity measures had different results but may reflect better repair in bowhead cells, more cell cycle delay in bowhead cells, or that the bowhead cells are undergoing more apoptosis, though this seems unlikely as the bowhead cells had a better survival than the human cells. Additional work is being done to determine the contribution of cell cycle arrest and DNA repair. This work was supported by the Maine Center for Toxicology and Environmental Health and NASA grant ACD FSB-2009.

1128 Kinetic and Mechanistic Assessment of Micronucleated Peripheral Blood Reticulocytes (MNRETs) in Rodents Using Flow Cytometry.


Evaluation by flow cytometry (FCM) increases the sensitivity and robustness of the in vivo rodent micronucleus assay. Kinetics of micronucleus induction can be assessed by FCM, since sample collection is not dependent upon terminal sacrifice, and only minimal amounts of blood are required. For example, the dose-dependent increase in mmRETs observed in Sprague-Dawley rats 24 hours after treatment with cyclophosphamide (CP) is undetectable by the 120-hour time point. These kinetics data support previous findings that circulating mmRETs can be detected in rats when proper sampling intervals are used. Mechanism of action (MoA) information can also be obtained by evaluating median channel fluorescence intensity (MFI) of mmRETs. As compared to vehicle controls, increases in MFI indicate an aneugenic MoA, while no change in MFI suggests a clastogenic MoA. In a MoA validation study, CD-1 mice were administered 5-amino- o-creosol (AOC), vincristine (VIN), or CP for 3 consecutive days, and blood samples were collected 24 hours after the last treatment. VIN and CP, but not AOC, induced dose-dependent increases in mmRETs. However, the MFI of the mmRETs increased only for animals treated with VIN, confirming the expected aneugenic and clastogenic MoAs for VIN and CP, respectively. In addition, MoA data are available immediately, without having to perform subsequent MoA analysis (as would be required after manual scoring), or conduct a separate study, which would increase animal use. Thus, use of FCM analysis and proper sampling times allows significant reduction in animal use by analyzing mmRETs within standard subchronic studies, as well as providing MoA information that would render follow-up studies unnecessary.

1130 Adequate Conditions for Performance of the Comet Assay Using 3-Dimensional Human Epidermal Model.

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To evaluate a risk assessment of genotoxicity when human skin is exposed to chemicals, we developed conditions for a comet assay using a 3-dimensional human epidermal model (LabCyte EPI-MODEL; LabCyte, Japan). Enzymes for cell suspension, a vehicle for insoluble chemicals, and several genotoxicants were investigated during protocol development. To this end, the test protocol was designed to obtain stable data on negative and positive controls using LabCyte. Each vehicle and test chemical solution was applied directly to the surface of the models and treated for 4 or 24 hours, then washed off. Cytotoxicity values were obtained using an MTT assay and were calculated at % tail in DNA as the endpoint of the comet assay. Other conditions and criteria were adapted from the alkaline comet assay protocol reported in the JaCVM International validation study. The results are summarized below. 1) Cells were detached by Liberase solution (Roche) or Trypsin solution (GIBCO), and more single cells could be efficiently retrieved using Trypsin solution. 2) The best vehicles for insoluble chemicals were not 100% DMSO or ethanol. The % tail in DNA treated with up to 25% DMSO or 2% ethanol in distilled water was low and had no effect. These aqueous solutions were adequate as negative controls and as vehicles for insoluble chemicals. 3) Mitomycin C (MMC), Methylmethanesulfonate (MMS), and 4-NQO (4-Nitroquinoline 1-Oxide) were investigated as potential positive controls. Of these genotoxicants, MMS showed stable data as a positive control. We established a practical, rapid and easy comet assay protocol using a 3-dimensional human epidermal model.

1131 Toxicity Study of an Ethanolic Extract of Acorus Calamus in Wistar Rats.

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Investigation was carried out to evaluate the safety of an extract of Acorus calamus Linn, rhizomes by determining its potential toxicity after acute and chronic administration in Wistar rats. For the acute toxicity study, ethanolic extract of Acorus calamus was administered to six female Wistar rats by oral gavage at dose levels of 175, 550, 1750 and 5000 mg/kg body weight according to OECD 425. Changes in general behavior, mortality and body weight were recorded for 14 days and were not performed observed nor were any mortality observed, but clinical signs like abdominal breathing, piloerection and tremors were observed in rats dosed with 1750 mg and 5000 mg/kg body weight of extract, immediately after dosing. The calculated LD50 was found to be more than 5000 mg/kg body weight. In the chronic toxicity study, the ethanolic extract of Acorus calamus was administered orally at doses of 0, 200, 400 and 600 mg/kg daily for 60 days in 20 male and 20 female Wistar rats. No mortality or changes in clinical signs and functional o-vissual observation (FOB) were noted but statistical significant reduction in body weight and feed consumption on day 29, 35, 42, 49, 56 and 60 were observed. No significant changes were observed in biochemical, hematological parameters and organ weights. The histopathological studies of important organs showed normal structure, suggesting no morphological alterations. After observing relatively high NOAEL values in the acute study in rats, and lack of mortality or clinically significant changes in the biochemical and hematological parameters in rats after 60 days of daily dosing, it may be concluded that the Acorus calamus extract does not appear to have significant toxicity. Thus, ethanolic extract of Acorus calamus rhizomes can be considered safe for the therapeutic use.


Gum Guggul Extract (GGE) is a mixture of several steroid-lipids isolated from the plant Commiphora mukul (gum guggul) of Indian origin, containing Z- and E-isomers of guggulsterone as major constituents. It has been used as Ayurvedic medicine to treat various disorders including bone fracture, arthritis, and inflammation for thousands of years. Currently the use of gum guggul as dietary supplements is...
increasing exponentially worldwide. There is sparse literature available to ade-
quately assess the safety to human health. Consequently GGE has been selected by the National Toxicology Program (NTP) for toxicological evaluation. In support of NTP studies a method was developed and validated for the assay of GGE in corn oil formulations. This effort also includes a 3-hour simulated dosing study, and a 42-day forward storage stability study of a formulation at ~5.0 mg/mL at different temperatures, along with an evaluation of a high dose formulation (~206 mg/mL) for homogeneity. The method uses high-performance chromatographic (HPLC) with UV detection and was validated for formulation of GGE in corn oil in the range of ~2.45 to ~40.7 mg/mL. The method met acceptance criteria for linearity, precision, and accuracy. The results obtained from a 42-day stability study on a formulation of ~5.0 mg/mL. GGE in corn oil shows that formulations can be stored under ambient, precision, and refrigerated, or freezer conditions for up to 42-days. The results of a 3-hour simulated dosing study for an ~5.0 mg/mL formulation, exposed to air and light, shows that the formulation is stable for 3 hours under simulated dosing conditions. At a high dose formulation of ~206 mg/mL GGE in corn oil was a sus-
pension and was evaluated for homogeneity; the results indicated that the formula-
tion was homogenous (0.7% RSD).

**1135 SAFETY ASSESSMENT OF AN ACAI-ENRICHED MIXED FRUIT AND BERRY BLEND (MONAVIE).**
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Monavie Active® (Salt Lake City, UT, USA) is a fruit and berry juice consumed in 15 countries. Using eukaryotic cells (Saccharomyces cerevisiae), Spada et al reported in 2008 that acai fruit (Euterpe oleracea Mart.) was mutagenic when tested in high concentrations. As Monavie contains acai as its predominant ingredient, a series US FDA compliant assays and tests using OECD protocols were performed in GLP labs: Ames test; gene mutation test in mouse lymphoma L5178Y cells; mi-

**1136 ELECTROCHEMICAL PROFLING USING COPPER NANOPORE-PLATED ELECTRODE FOR QUICK IDENTIFICATION OF OSTRICH MEAT AND EVALUATION OF MEAT GRADES.**
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Accurate identification of the origin of high-value meat species presents a consider-
abled challenge for government authorities and analysts. An HPLC method with specific selectivity and sensitivity to specific peptides was developed to differentiate ostrich meat from pork, beef and chicken, and to evaluate different grades and freshness of ostrich meat to assure fair pricing. Supernatant of centrifuged meat ho-
mogenates was filtered and directly injected for HPLC analysis. The Chromatographic separations were performed using phosphate buffer as mobile phase, in a portable flow-injection analysis system employing a copper nanopore-
icle-plated screen-printed electrode (Cun-SPE). The 4 meats could be differentiated in 5 minutes by characteristic chromatographic profiles consisting only 4 major peaks. Anserine was identified by LC/MS/MS as an avian-specific peak for differ-
entiation from mammal species and Carosine was proved to be the missing peptide for identification of Ostrich meat. Statistical analysis (ROC curve) demonstrated that peak ratios of ostrich meat could be used for evaluation of ostrich meat grades (price levels) with high sensitivity (up to 95%) and specificity (up to 100%). The effects of storage temperature and time on chromatographic profiles were studied for potential use of Cun-SPE to evaluate meat freshness. In conclusion, this HPLC-
EC method appeared to be superior to UV detection in terms of profile simplicity and devoid of derivatization or complex sample extraction procedures, therefore, rendering it a suitable method for routine rapid differentiation of ostrich meat from common low value species with the ability to simultaneously differentiate ostrich meat grades.

**1134 THE RELATIVE ROLES OF SELECTED STRESSORS IN THE PATHOGENESIS OF SUMMER SLUMP IN A RAT MODEL.**
The effects of fescue toxicosis are extremely costly to animal agriculture and are ex-
acerbated by heat stress, inducing "summer slump," characterized by reduced pro-
ductivity, as well as impaired thermoregulation. Some aspects of summer slump are direct effects of tall fescue endophyte toxins involving heat stress, while others are indirect effects of these stressors related to reductions in caloric intake. Rat models for summer slump have been developed and validated, and it was hypothesized that subacute or acute exposure to endophyte toxins, heat stress, and/or interactions as well as between these fescue toxicosis-associated stressors, play different roles in the pathogenesis of experimentally induced summer slump in male rats. Under thermoneutral or heat stress conditions, sexually mature male rats were ex-
posed for 15 days to diets designated as endophyte-free (E-), endophyte-infected (E+) or E+ pair-fed to endophyte-infected (E-PFE+), where intake of E- diet was re-
stricted to the rate of caloric intake in rats fed E+ diet. Body temperature, feed in-
take, weight gain, prolactin concentrations, weights of selected organs, and certain biochemical parameters were affected by toxin exposure, ambient temperature, and/or interactions between these stressors. Water and acid-base balance, sperm motility, and testicular morphology were primarily affected by heat stress. Serum testosterone concentrations and spermatogonial tend to be affected by only endo-

**1137 ANALYSIS OF DNA BINDING OF [3, 4-14C]-FURAN IN RAT LIVER BY ACCELERATOR MASS SPECTROMETRY.**
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Furan, which has been shown to be present in a variety of heated foods, is a potent hepatotoxic and liver carcinogen in rodents, but the mechanism of furan carcino-
genicity is still unclear. Furan is metabolised by cytochrome P450 enzymes to cis-2-
butene-1,4-dial, which has been shown to react with 2'-deoxyribonucleosides in vitro. However, standard genotoxicity tests with furan have generated inconsistent results, and the important question whether or not furan forms DNA adducts in vivo has not been fully resolved. To assess DNA binding of furan in rat liver and to characterize potential DNA adducts, male F344 rats (n = 5) were administered single oral doses of 0, 0.1 or 2.0 mg/kg bw [3,4-14C]-furan (20 mCi/mmol) and were sacrificed 2 h later. DNA from liver (target) and kidney (non-target organ) was extracted by ion exchange chromatography and analyzed by accelerator mass spectrometry (AMS). The 14C-content in DNA was significantly increased in a dose-dependent manner, ranging from 7.8 ± 3.5 amol 14C/μg DNA (corresponding to 16.5 ± 7.4 adducts/105 nucleotides) to 153.3 ± 100.2 amol 14C/μg DNA (corresponding to 3.3 ± 2.1 adducts/107 nucleotides) in livers of rats treated with 0.1 and 2.0 mg/kg bw, respectively. To discriminate between DNA binding and metabolic incorporation, liver DNA was enzymatically hydrolysed, fractionated by HPLC-DAD and the radiocarbon content of each fraction was analysed by AMS. The majority of 14C-label was not associated with normal nucleotides, suggesting that the increase in 14C-content in DNA extracted from livers of rats treated with [3,4-14C]-furan is primarily due to covalent DNA binding. In contrast, radioactivity was found to elute at similar retention times as synthesized DNA adduct standards of 2'-deoxyadenosine and 2'-deoxyctydine. Current work is aimed to elucidate the structure of the furan-derived DNA adducts. This work was supported by FP6 of the European Union (SSPE-CT-2006-44393).

1138 IDENTIFICATION OF TARGET PROTEINS OF FURAN IN RAT LIVER.

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Furan was recently found to be present in a variety of food items that undergo heat treatment. Furan is a potent hepatotoxic and liver carcinogen in rodents, but the mechanisms of furan-induced tumor formation are poorly understood. In order to assess the role of protein binding of furan in furan toxicity and carcinogenicity, male F344 rats were administered [3,4-14C]-furan at doses of 0, 0.1 and 2.0 mg/kg bw and sacrificed after 2h. Liquid scintillation counting of protein extracts revealed a dose-dependent increase in the amount of 14C bound to liver proteins, with 9.3 ± 2.2 fmol 14C/μg protein and 91.7 ± 8.1 fmol 14C/μg protein in low and high dose animals, respectively. After separation of liver proteins by two-dimensional gel electrophoresis and subsequent detection of radioactive spots by fluorography, target proteins of reactive furan intermediates were identified by ESI-QTOF-MS/MS and database search via Mascot. Fluorography of gels representing pH ranges 3–11, 4–7 and 6–9 consistently revealed - 50, 37 and 17 discrete protein spots containing radioactivity, respectively. The target proteins identified so far are located in various cellular compartments and mainly represent enzymes that primarily take part in lipid metabolism, glucose metabolism and cell death. Interestingly, several of the proteins identified, i.e. 3-alpha-hydroxysteroid dehydrogenase, alpha 2u globulin, alpha enolase, long-chain-fatty-acid-CoA ligase, malate dehydrogenase, ribonuclease UK114, thioridoxin and triosephosphat isomerase were previously shown to be targets of other compounds known to cause toxicity via reactive metabolite formation such as mycophenolic acid and tetracin A. More detailed biological interpretation will provide information as to how inactivation of protein function through covalent binding of furan reactive metabolites may contribute to the mechanisms of furan toxicity and carcinogenicity. This work was supported by DFG (MA 3323/3-1) and FP6 of the European Union (SSPE-CT-2006-44393).

1139 DIETARY SUPPLEMENT-DRUG INTERACTION: DEVELOPMENT OF P450 INHIBITION PROFILE METHOD.

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It is estimated that about one in four patients taking prescription medication also take a dietary supplement and may be at risk of potential drug-supplement interactions. It is estimated that about one in four patients taking prescription medication also take a dietary supplement and may be at risk of potential drug-supplement interactions. The drug side effects and toxicity and often the drug efficacy may be altered by the interaction of dietary supplement with drug metabolizing cytochrome P450 (CYP) enzymes. There are many reports describing the effect of dietary supplement on CYP activities, however majority of these are reported as the effect on single CYP. Therefore, we evaluated the multiple CYPs inhibition profile of dietary supplement, for estimation of drug interaction as preliminary assay. We investigated effects of herbal extracts and chemical components in dietary supplement ingredients on human liver CYP3A4, 2D6, 1A2, 2C9, 2C19, and 2E1 activities. The assay was performed using pooled human microsome and cocktail substrate, midazolam (3A4), butafural (2D6), phenacetin (1A2), diclofenac (2C9), methylenephen (2C19), and chlooroxazone (2E1). The metabolite products of each substrate were measured by LC/MS/MS. Strong inhibition of CYPs activities was noted with St. John’s worth (SJW), Ginkgo extract, extract. Most of the herbal extracts were found to contain high levels of polyphenol. As hyperforin that was one of the chemical components of SJW showed inhibition to all CYPs, it might contribute to the inhibitory activities of SJW. In addition quercetin found in Ginkgo, also showed strong inhibition. The work is in progress to evaluate the effects of several food ingredients on CYP.

1140 ASSESSMENT OF THE NEPHROTOXICITY OF A SEVEN-DAY COMBINED-EXPOSURE TO MELAMINE AND CYANURIC ACID IN F344 RATS.

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In 2007 the intentional adulteration of wheat flour, used in the preparation of pet food, with melamine and a number of derivatives, including cyanuric acid, caused renal failure and death of a substantial number of cats and dogs. While literature data indicate that melamine and cyanuric acid, by themselves, have low toxicity in a range of mammalian species, recent evidence demonstrates that the co-exposure to low levels of both compounds elicits nephrotoxicity due to the formation of melamine cyanurate crystals in the kidney nephrons. To determine the approximate levels of co-exposure capable of eliciting nephrotoxicity, we exposed groups of male and female F344 rats to target doses of 200 mg/kg BW/day melamine or cyanuric acid or 0, 1, 3.3, 10, 33, or 100 mg/kg BW/day melamine and cyanuric acid in the diet for seven days. No toxicity was observed in the rats exposed to the individual compounds, whereas marked toxicity was observed in the animals treated with 33 or 100 mg/kg BW/day melamine and cyanuric acid. The kidneys were pale-yellow and enlarged, with melamine-cyanurate-like crystals in the tubules of both the 33 or 100 mg/kg BW melamine and cyanuric acid dose groups. A statistically significant increase in BUN and serum creatinine levels was also registered in these groups. No significant changes were detected in the remaining combined treatment groups, indicating that under these experimental conditions the threshold of exposure capable of eliciting nephrotoxicity was between 10 and 33 mg/kg BW/day. The results from this study confirm the synergistic nephrotoxic effect of melamine and cyanuric acid, and support the hypothesis that the toxicity is determined by the co-precipitation of melamine and cyanuric acid as melamine cyanurate in the nephron.

1141 INFLUENCE OF DIETARY STEROL EXPOSURE ON HEPATIC OXysterol PROFILES AND GENETIC STABILITY.

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Phytosterols (PS) such as β-sitosterol (SitOL) are found at high concentrations in functional food designed to reduce serum cholesterol (ChOL) levels. Due to their unsaturated structure, both ChOL and SitOL can react to various oxysterols which are associated with adverse health effects. In order to investigate the correlation of oxysterols with genetic stability, we investigated the levels of oxysterols (i) in the livers of female guinea pigs fed different diets containing SitOL and/or ChOL for 2 weeks as well as (ii) in the diet and compared them with the micronuclei (MN) rate in erythrocytes. Oxysterols were isolated by liquid phase purification, extracted by solid phase extraction and analyzed by GC/MS. In order to quantify the oxysterol content, corresponding deuterium-labeled internal standards were used. The MN rate of blood smears was analyzed by light microscopy after staining with May-Grünwald/Giemsa solution. In the livers of the guinea pigs, the following oxysterols were detected: 5β-epoxy-ChOL (0.6 ± 0.2 up to 1.5 ± 0.4 μg/g liver, depending on the diet) > 7-keto-ChOL > 5β,6-epoxy-ChOL > 7-HO-ChOLs > β/γ-HO-SitOL > 7α-HO-SitOL (0.02 ± 0.01 up to 0.04 ± 0.01 μg/g liver, depending on the diet). In the livers of the ChOL (0.2%) only group, statistically higher levels of 7-HO-ChOLs and 7-keto-ChOL were detected than in those fed ChOl (0.2%) together with PS-rich (0.8%) diets. Furthermore, the MN rate was higher in the ChOL (0.2%) only group than in the other groups. In contrast, there was no indication of an association of 7-HO-SitOLS with MN level. While relative concentrations of oxysterols in the diets corresponded to those in the livers, oxysterols were probably taken up via diet. In conclusion, dietary uptake of oxysterols is reflected in their tissue levels. Oxidation products of ChOL may affect genetic stability and may thus have an impact on human health whereas PS seem to reduce not only hepatic ChOL but also oxy-ChOL levels.
UC-II alone is significantly more effective than glucosamine+chondroitin or UC-II+glucosamine+chondroitin (Group-IV) showed significant pain reduction in arthritic pain (30% and 28%, respectively). Similarly, horses receiving a combination of UC-II, 480mg, Group-III (glucosamine HCl and chondroitin sulfate, 5.4g and 1.3g, respectively, twice daily for the first month and thereafter once daily), and Group-IV (UC-II+glucosamine+chondroitin) daily for a 5 month period. On a monthly basis, horses were examined for overall pain (scale 1-10), pain upon limb manipulation using flexion test (scale 0-4), physical condition, and hepatic and renal functions. The arthritic condition of Group-I horses (placebo) remained unchanged throughout the study period, while those receiving UC-II (Group-II) showed significant reduction in overall pain from 5.7±0.42 (100%) to 0.7±0.42 (12%); and pain upon limb manipulation from 2.3±0.37 (100%) to 0.5±0.18 (22%). Glucosamine and chondroitin treated horses showed significant reduction in arthritic pain (30% and 28%, respectively). Similarly, horses receiving a combination of UC-II+glucosamine+chondroitin (Group-IV) showed significant pain reduction (57% and 50%, respectively). Physical condition (body weight, temperature, respiration and heart rate), and hepatic (GGT, ALP and bilirubin) and renal (BUN and creatinine) functions remained unchanged, suggesting that these supplements were well tolerated and safe. These results indicate that comparatively UC-II alone is significantly more effective than glucosamine+chondroitin or UC-II+glucosamine+chondroitin in ameliorating arthritic pain in horses.

DEOXYNIVALENOL INGESTION PREVENTS AND AMELIORATES DIET-INDUCED OBESITY IN THE MOUSE.

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Obesity is a growing pandemic in need of prevention and intervention strategies. Here we tested the hypothesis that low dose dietary exposure to the trichothece deoxynivalenol (DON) prevents and ameliorates diet-induced obesity (DIO) in adult female B6C3F1 mice. When fed a high fat diet (HFD) containing 0.2, 5 and 10 ppm DON for 10 weeks (preventive model), mice fed 5 ppm and 10 ppm exhibited a 15% and 24% weight suppression and a 50% and 83% reduction in peri-uterine fat, respectively. When fed HFD for 8 weeks to induce obesity and then fed 0, 2, 5 and 10 ppm DON in HFD for 8 weeks (therapeutic model), mice fed 5 ppm and 10 ppm exhibited a 16% and 23% weight suppression and a 0% and 40% reduction in peri-uterine fat, respectively. In the preventive model, DON at 5 ppm and 10 ppm suppressed circulating levels of insulin like growth factor acid labile subunit by 18 and 30% and by 20% and 42% in the therapeutic model, respectively. In a follow-up study, food consumption was measured in 0 and 10 ppm DON groups prior to and after the switch from HFD to HFD containing DON. Within 1 day of exposure, DON-fed mice ate significantly less than the control, while significant weight loss was seen after 6 days. Taken together, our data show that DON exposure prevents and ameliorates diet-induced obesity in mice and this corresponds to both alterations of the growth hormone axis and decreased food consumption.

THEORETICAL AND SAFETY EVALUATIONS OF UC-II OR GLUCOSAMINE PLUS CHONDROITIN OR UC-II, GLUCOSAMINE AND CHONDROITIN COMBINATION IN OSTEOARTHRITIC HORSES.

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Millions of horses around the world suffer from arthritis. Of the two common forms of arthritis (osteoarthritis, OA; and rheumatoid arthritis, RA), OA often affects horses due to multiple reasons. The present investigation was conducted on four groups of horses treated as follows: Group-I (placebo), Group-II (UC-II, 480mg), Group-III (glucosamine HCl and chondroitin sulfate, 5.4g and 1.3g, respectively, twice daily for the first month and thereafter once daily), and Group-IV (UC-II+glucosamine+chondroitin) daily for a 5 month period. On a monthly basis, horses were examined for overall pain (scale 1-10), pain upon limb manipulation using flexion test (scale 0-4), physical condition, and hepatic and renal functions. The arthritic condition of Group-I horses (placebo) remained unchanged throughout the study period, while those receiving UC-II (Group-II) showed significant reduction in overall pain from 5.7±0.42 (100%) to 0.7±0.42 (12%); and pain upon limb manipulation from 2.3±0.37 (100%) to 0.5±0.18 (22%). Glucosamine and chondroitin treated horses showed significant reduction in arthritic pain (30% and 28%, respectively). Similarly, horses receiving a combination of UC-II+glucosamine+chondroitin (Group-IV) showed significant pain reduction (57% and 50%, respectively). Physical condition (body weight, temperature, respiration and heart rate), and hepatic (GGT, ALP and bilirubin) and renal (BUN and creatinine) functions remained unchanged, suggesting that these supplements were well tolerated and safe. These results indicate that comparatively UC-II alone is significantly more effective than glucosamine+chondroitin or UC-II+glucosamine+chondroitin in ameliorating arthritic pain in horses.

CHROMIUM(III) COMPLEX: CHROMIUM DINOCYSTEINATE (CDNC).

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Few trivalent chromium(III) complexes has been demonstrated to improve protein, fat and glucose metabolism. It also helps in energy production and the promotion of lean body mass and muscle integrity. In previous studies, Zucker diabetic fatty (ZDF) rats were supplemented with CDNC (400 µg elemental chromium(III)/kg body weight/day) over a period of 8 weeks. CDNC significantly lowered blood glucose (24%), HbA1c (11%), CRP (20%), MCP-1 (49%), ICAM (10%) and lipid peroxidation (20%), respectively, compared to control rats. This study was focused on determining the broad spectrum safety of CDNC in a number of animal models including: acute oral, acute dermal irritation, primary eye irritation toxicity studies, mouse micronucleus assay and Ames’ bacterial reverse mutation assay. A 90-day repeated dose oral gavage toxicity study was also conducted where rats were administered either 0, 0.25, 2.3 or 5.7 mg/kg/day CDNC and sacrificed on 0, 30, 60 or 90 days of treatment. Body weight, food and water consumption, selected organ weights as such as and percentages of body and brain weight, ocular health, hematology, blood chemistry, clinical chemistry and histopathology were assessed. In acute oral and acute dermal toxicity studies, LD50 of CDNC were found to be >2000 mg/kg body weight in Sprague-Dawley rats. The primary skin irritation study in New Zealand Albinob rabbits demonstrated CDNC as slightly irritating. The eye irritation study exhibited that CDNC is severely irritating. Ames’ bacterial reverse mutation assay and mouse micronucleus assay demonstrated CDNC‘s 90 non-mutagenic. The 90-day repeated dose oral gavage toxicity study demonstrated no significant toxicity of CDNC. No abnormal changes were observed at any time points. These results demonstrate the broad spectrum safety of CDNC.
The objective of this study was to evaluate the safety of an eicosapentaenoic acid (EPA)-rich oil produced from genetically modified *Yarrowia lipolytica* yeast in a 90-day rat study. Groups of 20 male and female rats/sex/group were dosed with olive oil (control) or EPA oil by daily oral gavage, either undiluted (High EPA: 976 mg EPA/kg/day) or diluted in olive oil (Medium EPA: 488 mg EPA/kg/day or Low EPA: 98 mg EPA/kg/day). Additional groups received either a reference GRAS (generally recognized as safe) fish oil (513 mg EPA/kg/day) or deionized water. All rats were evaluated for standard in-life and anatomic pathology parameters. Ten rats/sex/group received neurobehavioral and clinical pathology evaluations, and 5 rats/sex/group had fatty acids measured in plasma. Changes in serum lipids were observed including lower total serum cholesterol in male and female High EPA and fish oil groups (largely due to lower non-HDL cholesterol); lower total and non-HDL cholesterol in medium EPA females; and lower HDL in High EPA and fish oil males. Liver weights were increased in High EPA and Medium EPA (female only) groups with non-associated liver clinical or histopathology. Nasal microscopic lesions attributed to regurgitation of test substance were observed in High and Medium EPA and fish oil groups. No adverse test substance-related effects were observed on body weight, nutritional, neurobehavioral, or other clinical or anatomic pathology parameters. In conclusion, exposure to the EPA-rich oil produced from yeast for approximately 90 days did not produce adverse effects at daily doses up to 976 mg EPA/kg/day. The overall safety profile of the EPA-rich oil was comparable to that of GRAS fish oil.

The potential developmental toxicity and genotoxicity of eicosapentaenoic acid (EPA) oil produced from genetically modified *Yarrowia lipolytica* yeast was assessed in a series of safety assessment studies. To assess developmental toxicity, groups of 22 time-mated rats were dosed with EPA oil administered neat or diluted with olive oil to deliver doses of approximately 98, 488, or 976 mg/kg/day of EPA. The control group was dosed with olive oil. Doses (3 mL/kg) were administered once daily by gavage on gestation days (GD) 6-20. During the in-life portion of the study, maternal clinical observations, body weights, and food consumption data were collected. On GD 21, all dams were euthanized and a gross external and visceral examination was performed. The uteri of each pregnant female was removed and the uterine contents were examined, and the fetuses were removed and individually identified, weighed, sexed, and examined for external, visceral, head, or skeletal alterations. There was no evidence of maternal toxicity at any dose level tested. Maternal body weights, food consumption, clinical observations, and gross postmortem observations data were comparable across all groups. No developmental toxicity was observed at any dose level. The mean numbers of corpora lutea, implantation sites, resorptions, and live fetuses were comparable across all groups as were group means for litter sex ratio, fetal weight, and incidences of fetal malformations and variations. Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 976 mg/kg/day, the highest dose tested. The EPA oil was also evaluated for safety in three in vitro and in vivo genotoxicity tests. The oil was not mutagenic in vitro in the bacterial reverse mutation or mouse lymphoma assay, and was not clastogenic in an in vitro micronucleus study in mice.
Ingestion of genetically modified (GM) foods has been alleged to cause various adverse effects in laboratory animals, including changes at the cellular level. We evaluated studies reporting ultrastructural and molecular modifications of nuclei in hepatocytes and pancreatic acinar cells of animals fed a diet containing GM soybeans. A total of six studies were identified; because five of these were from a single laboratory using similar research methods and reporting similar findings, they were examined as a whole. Our analysis found that the reviewed studies suffer from many methodological deficiencies, the most critical of which are: (1) study designs that fail to control for possible litter effects; (2) inadequate methodological procedures to ensure an unbiased, quantitative assessment (e.g., principles of stereologic morphometry are not followed); (3) inappropriate statistical methods; and (4) failure to address potential confounding, especially that due to differences in the phytoestrogen content of the control and GM soybean–based feeds. Based on these shortcomings and the nature of the observations reported, conclusions regarding the potential impact of GM soybean-based diets on the subcellular morphology of selected organs are not supported by the reviewed studies. Because it only evaluates small amounts of tissue, and thus, cannot be viewed as representative of effects in the whole animal, use of electron microscopy is not recommended in OECD testing guidelines for assessing the potential toxicity of chemicals. If histopathological findings are observed in safety studies, however, such methods may be pursued to further elucidate a chemical's toxic mode of action. Procedures for the proper nutritional assessment of GM foods also do not rely upon the use of electron microscopy. To date, adverse histopathological findings have not been reported in animal toxicity studies of GM soybeans. Thus, the electron microscopy studies reviewed herein are not suitable for use in a risk assessment context.

PS 1155 TOXICOLOGY STUDIES WITH N-ACETYLGLYCINE.

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N-acetylglycine (NAGly) has been identified as a minor constituent of numerous foods. The current postuer reports the outcomes of in vitro and in vivo genotoxicity, acute oral and repeated dose dietary toxicity studies conducted with NAGly. No evidence of genotoxicity was observed with NAGly in in vitro bacterial tester strains or in in vivo bone marrow micronucleus studies conducted in mice. No mortalities or evidence of adverse effects were observed in Sprague-Dawley rats following acute oral gavage with NAGly at a dose of 2000 mg/kg of body weight or following repeated dose dietary exposure to NAGly at targeted doses of 100, 500, or 1000 mg/kg of body weight/day for 28 days. No biologically significant or test substance related differences were observed in body weights, feed consumption, or clinical pathology response variables in any of the treatment groups. Based on these results it was concluded that NAGly is not genotoxic or acutely toxic. Further, the no-observed-adverse-effect-level (NOAEL) for systemic toxicity from repeated dose dietary exposure to NAGly was 898.9 mg/kg of body weight/day for male rats and 989.9 mg/kg of body weight/day for female rats.
Exposure to gluten in individuals with CD results in an immune-mediated enteropathy which involves morphological damage to the lining of the small intestine. A diverse array of clinical signs and symptoms are often associated with gluten exposure to CD sufferers. Avoidance of gluten is the principal method of management of these adverse effects. Thus, the determination of a tolerable daily intake level of gluten in sensitive individuals is relevant. A health hazard assessment of the development of morphological and clinical adverse effects of CD was performed. All available published studies that performed oral food challenge tests in individuals susceptible to the development of CD and that included employing appropriate uncertainty factor(s). The TDI for morphological effects associated with CD is 0.4 mg gluten/day. The morphological TDI for gluten was based on results from subchronic challenge tests. This data set best reflected the lower limits of reactivity to gluten because of limitations that exist in the low-dose challenge data currently available for acute and chronic gluten exposures. The TDI for clinical effects in those with CD is 0.015 mg gluten/day. The clinical TDI derived were the same for each exposure duration because the adverse effects levels found in challenge studies were similar across each duration of exposure. In summary, the estimated TDIs for gluten in individuals with CD are at a less than 1 mg level for morphological and for clinical adverse effects.

**1157 DIETARY FACTORS THAT INFLUENCE KIDNEY CALCINOSIS (KC) IN WEANLING, FEMALE SPRAGUE-DAWLEY RATS.**


Purified ingredient diets have historically promoted HC due to excess calcium (Ca) deposition, whereas most grain-based chow diets (chows) limit its development. Diets with a Ca to phosphorus (P) molar ratio <1 can promote KC in weanling female rats. The AIN-93G purified diet doesn’t promote KC due likely to Ca:P >1, but at the expense of having a P deficient mineral mix reliant on casein to supply recommended P for rodent growth. Data also suggest that dietary carbohydrate, fiber type, and sulfur amino acid supplement (i.e. L-Cysteine [L-Cys] or DL-methionine [DL-Met]) can affect KC. We studied the contributions of these factors (separately or all in combination) on KC in the context of a diet with P sufficient mineral mix and Ca:P >1. Weanling, female Sprague-Dawley rats were fed purified diets with Ca:P =1.1 for 28 days after which kidneys were analyzed for Ca using ICP-MS. Diet 1 contained corn starch/dextrose as the carbohydrate, inulin/cellulose as the fiber and L-Cys as the supplemental sulfur AA. We sequentially examined the effects of replacing starch with sucrose (Diet 2), inulin with cellulose only (Diet 3), L-Cys with DL-Met (Diet 4), or the combination of all 3 factors (Diet 5). The AIN-76A historically induces KC and so served as a positive treatment. The AIN-93G diet and 2 grain-based diets (NIH-31M, Purina 5002 [5002]) served as negative treatments. As expected, the AIN-76A diet had the highest average renal Ca levels among all groups (mean =14.2 mg/g) while Diet 1, 5002, and AIN-93G all had similar levels (0.2–0.3 mg/g). Replacing starch with sucrose tended to elevate kidney Ca by 6-8-fold relative to Diets 1, 3, and 4. Diet 5 (with all 3 factors) increased kidney Ca further (2.5-fold from Diet 2 and 21-fold from Diets 3 and 4). NIH-31M tended to raise kidney Ca relative to rats fed control (6-fold), 5002 (6-fold) and AIN-93G (10-fold). These data suggest that 1) Ca:P >1 maintains normal kidney Ca within a P sufficient mineral mix, 2) carbohydrate type influences KC in weanling female rats when the dietary Ca:P molar ratio >1 and 3) a grain-based diet can promote moderate KC.

**1158 PHARMACOKINETICS OF MELAMINE IN A RUMINANT MODEL.**

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In March of 2007 a wide spread pet food recall resulted due to renal failure in cats and dogs, believed to be caused from food produced with proteins contaminated with melamine. Shortly after the US FDA prevented foods containing melamine that had been fed this recalled dog food from entering the market. A year later Chinese officials reported that an estimated 300,000 infants had experienced kidney problems after drinking infant formula tainted with melamine. Although the feeding of saladage dog food to food animals is only for non-ruminants, melamine is a known metabolite of the pesticide cromazine and has been found to be metabolized both by mammals and by plants themselves, potentially leading to ruminant ingestion by grazing on plants treated with the pesticide. Melamine is a 1-3-5 triazine that can undergo further hydrolysis into ammonia, ammelide, and finally cyanuric acid. Two dairy goats were orally gavaged with a dose of 40mg/kg melamine in water, then milk and blood samples taken for 72 hours to determine the concentration and residence time of melamine. Samples were analyzed by HPLC –UV and UPLC-MS to determine the concentrations of melamine (and possible metabolites) in milk and plasma. Maximum milk concentrations (10.37 μg/mL) occurred 12 hours post dose, with an estimated milk half-life of 3.15 hours. The milk was found to be below the limit of detection (LOD of 0.05μg/mL) after 72 hours. Maximum plasma concentrations (6.92 μg/mL) were also found 12 hours post dose of the plasma half-life was estimated to be 5.94 hours. These data suggest that melamine may be absorbed, distributed, and/or cleared differently in ruminants when compared to monogastrics. Ongoing studies are looking to determine if ammeline, ammelide, or cyanuric acid are present in either the milk or plasma and whether these contaminants can be relayed in meat and/or milk from ruminants to the human food chain.

**1159 EXTRUSION COOKING USING A TWIN-SCREW APPARATUS REDUCES TOXICITY OF FUMONISIN-CONTAMINATED CORN GRITS.**

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Extrusion cooking using a single screw configuration reduced fumonisin concentrations of corn grits in an earlier study. Adding glucose before cooking enhanced reductions and, in one of three trials, partially reversed in vivo toxicity. To determine the effectiveness of extrusion using the more efficient twin-screw configuration, batches of Fusarium verticillioides–fermented corn grits (Batch-1 = 9.7 ppm fumonisin B1, FB1, B1), Batch-2 = 50 ppm FB1, B1, determined by HPLC were extruded (Batch-1E, Batch-2E) or extruded with 10% w/w glucose (Batch-1EG, Batch-2EG). FB1 concentrations of the cooked batches were: Batch-1E = 2.7; Batch-1EG = 0.6; Batch-2E = 18; and Batch-2EG = 5.7 ppm. These values correspond to FB1, reductions of 72%, 94%, 64% and 89%, respectively. The above batches and uncooked (control) or extruded (extrusion control) uncontaminated (<0.2 ppm FB1) grits were mixed (1:1) with basal chow and fed to male rats for 3 (n=5/group) or 8 (n=5/group) weeks. Differences in general appearance, body weights or hematological and serum chemistry profiles were not found. Evidence of toxicity was limited to the kidneys. Relative kidney weights were decreased in rats fed Batch-1 or Batch-2 and apoptotic lesions typically caused by fumonisins were found in the Batch-1, Batch-1E, Batch-2, Batch-2E, and Batch-2EG groups. They were most severe in those groups fed diets having the highest FB1 concentrations: that is, Batch-1 (4.9 ppm), Batch-2 (25 ppm) and Batch-2E (9.0 ppm). Minimal to mild lesions were found in groups fed Batch-1E (1.4 ppm) or Batch-2EG (2.9 ppm) while control kidneys from rats fed Batch-1EG (0.32 ppm) were grossly remarkable upon microscopic examination. Together, the findings indicate that extrusion with glucose using a twin-screw configuration reduces the concentrations and toxicity of FB1, in contaminated corn grits.

**1160 IN VITRO EVALUATION OF NOVASIL CLAY FOR REDUCTION OF AFLATOXIN B1 IN COMMON GHANAIAN FOODS.**

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Aflatoxin B1 (AFB1) has been implicated in the etiology of acute and chronic disease in humans and animals. Cost effective and culturally acceptable intervention strategies for the elimination of dietary AFB1 are of critical need in populations at high risk for aflatoxosis. Previously our laboratory has demonstrated an encapsulated calcium montmorillonite clay, Novasil (NS), to be safe and efficacious in decreasing AFB1, biomarkers of exposure in a Ghanaian population. A planned pilot study in the same population will determine the efficacy of NS incorporated into commereal consumed at each meal. Here, preliminary studies were performed to determine the stability of the NS-AFB1 complex through the cooking and fermentation process of common Ghanaian foods. Additionally, the ability of NS to sequester AFB1 from a corn matrix was also evaluated. Sorption of AFB1 was assessed in batches of common Ghanaian foods. Additionally, the ability of NS to sequester AFB1 from a corn matrix was also evaluated. Sorption of AFB1 was assessed in batches and uncooked (control) or extruded (extrusion control) uncontaminated (<0.2 ppm FB1) grits were mixed (1:1) with basal chow and fed to male rats for 3 (n=5/group) or 8 (n=5/group) weeks. Differences in general appearance, body weights or hematological and serum chemistry profiles were not found. Evidence of toxicity was limited to the kidneys. Relative kidney weights were decreased in rats fed Batch-1 or Batch-2 and apoptotic lesions typically caused by fumonisins were found in the Batch-1, Batch-1E, Batch-2, Batch-2E, and Batch-2EG groups. They were most severe in those groups fed diets having the highest FB1 concentrations: that is, Batch-1 (4.9 ppm), Batch-2 (25 ppm) and Batch-2E (9.0 ppm). Minimal to mild lesions were found in groups fed Batch-1E (1.4 ppm) or Batch-2EG (2.9 ppm) while control kidneys from rats fed Batch-1EG (0.32 ppm) were grossly remarkable upon microscopic examination. Together, the findings indicate that extrusion with glucose using a twin-screw configuration reduces the concentrations and toxicity of FB1, in contaminated corn grits.
EVALUATION OF AFLATOXIN B1 ADSORPTION CAPACITY OF EDIBLE CLAYS FROM THE MARKETPLACE IN GHANA.

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Seventy edible clays from the Marketplace in Ghana were analyzed by equilibrium isothermal analysis to evaluate their adsorption capacities for aflatoxin B1 (AFB1). The clays were from the Aschanti, Greater Accra and Brong Ahafo regions. Uniform Particle Size NovaSil (UPSN) from BASF and a Na-bentonite from AMCOL Speciality Minerals were used as controls for AFB1 adsorption. X-Ray Diffraction (XRD) analysis was used to verify the mineral identity of controls and minerals present in clays from Ghana. AFB1 isotherms were run at pH 6.5 in triplicate using 11 different concentrations of aflatoxin of 0.1 mg of each clay, while water and AFB1 (8 µg/ml) were used as controls. Computer-generated isotherm data were analyzed and the capacity (Qmax) and affinity (KF) of adsorption were calculated. According to the sorption pattern of AFB1, on the clay surfaces, clays were classified in 6 different categories: H, L, C, S, hybrid H2-C2 and non-defined sorption pattern. UPSN and Na-bentonite showed an L-shape sorption pattern with Qmax values of 0.4 and 0.3 mol/kg AFB1, respectively. The most effective sample from Ghana had Qmax = 0.07, and the least effective had Qmax = 0.00. None of the Ghanaian clays were comparable with NovaSil or Na-bentonite; hence they would not be expected to decrease the bioavailability of AFB1. The XRD patterns of representative samples from Ghana (12) showed peaks of kaolinite, mica, feldspars (albite) and quartz. Some samples showed a possible smectite peak, although the peak was smaller than in controls. XRD patterns for UPSN displayedmontmorillonite peaks; while peaks of montmorillonite and gypsum were observed in the Na-bentonite. In conclusion, the clays from Ghana did not effectively bind AFB1 when samples from Ghana (12) showed peaks of kaolinite, mica, feldspars (albite) and...
contrast to VGSC activators such as brevetoxin and antillatoxin, we found that hoiamide A suppressed neurite outgrowth in neocortical neurons with an IC50 value of 4.89 nM. This action of hoiamide A is three orders of magnitude more potent than that as a sodium channel partial agonist. Further study demonstrated that hoiamide A increased LDH efflux, produced nuclear condensation and stimulated caspase-3 activity with EC50 values of 3.66, 2.55 and 4.33 nM, respectively. These data indicate that hoiamide A triggers neuronal death in neocortical neurons by both necrotic and apoptotic mechanisms. Further pharmacological evaluation excluded VGSCs, AMPA/kainate receptors, NMDA receptors and Na+-Ca2+ exchanger as potential targets for hoiamide A-induced neurotoxicity. Both PKC and PLC signaling pathways were also excluded. The broad-spectrum caspase inhibitor, Z-VAD-FMK, did however completely inhibit hoiamide-A induced LDH efflux and nuclear condensation. Additionally, a JNK inhibitor, but not ERK and p38 inhibitors, attenuated hoiamide A-induced LDH efflux and nuclear condensation. Hoiamide A treatment was found to stimulate JNK phosphorylation in neocortical neurons and nuclear condensation. Additionally, a JNK inhibitor, but not ERK and p38 inhibitors, attenuated hoiamide-A induced LDH efflux and nuclear condensation. Further study demonstrated that Z-VAD-FMK, did however completely inhibit hoiamide-A induced LDH efflux and nuclear condensation. Additionally, a JNK inhibitor, but not ERK and p38 inhibitors, attenuated hoiamide-A induced LDH efflux and nuclear condensation. Hoiamide A treatment was found to stimulate JNK phosphorylation in neocortical neurons and nuclear condensation. Additionally, a JNK inhibitor, but not ERK and p38 inhibitors, attenuated hoiamide-A induced LDH efflux and nuclear condensation.

**1166 EXPOSURE TO ACROLEIN CAUSES PLATELET ACTIVATION.**

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Acrolein is a toxic aldehyde generated during combustion of fossil fuels and is found in high concentration in cigarette smoke. Acrolein is also a natural food constituent and is generated in overheated fat-containing foods or oils. We and others have previously showed that acrolein causes cancers. Since thrombosis is an underlie cause of several cardiovascular disease, in the present study, we examined the effect of acrolein exposure on platelet activation. C57BL/6 male mice were exposed to 1 - 5 ppm acrolein for 6h/day for 1-4 days or gavage-fed with 1 - 5 mg/kg acrolein for 24-48 hours, and platelet activation was measured. With both modes of exposure, the ADP-induced platelet aggregation was significantly augmented in acrolein-treated animals both in the platelet rich plasma and in the washed platelets, as compared with their respective controls. Acrolein-induced hyper platelet aggregation was observed as early as 4h after oral exposure or 6h after inhalation exposure. Acrolein-induced hyper platelet aggregation was sustained for 72h after the oral exposure. Exposure to acrolein significantly increased the fibrinogen binding to platelets (P<0.01), however, surface expression of CD41 in acrolein-exposed mice was not affected. Exposure to acrolein did not affect platelet glutathione concentration but increased protein-acrolein adduct formation (P<0.01). Platelet-leukocyte aggregates were increased by 2-3 fold in acrolein-exposed mice. Concentration of platelet factor 4 in the plasma of acrolein-exposed mice was increased by 1.6-2.7-fold. Clotting time, after clipping a small section of the tail, was decreased by 30% in acrolein-exposed mice. These data suggest that exposure to acrolein hastens the overall coagulation process and potentially induces a pro-thrombotic state in mice.

**1167 IMPACTS OF INSECT DAMAGE ON FUNGAL INFECTION, FUMONISIN LEVELS, AND GRAIN QUALITY FOR ETHANOL PRODUCTION IN BT AND NON-BT MAIZE HYBRIDS.**

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In the northern hemisphere, maize is the primary substrate for ethanol production. Dried distiller’s grains and solubles (DDGS) are a major co-product of the ethanol industry, providing a rich feed supplement for livestock. Revenue from DDGS sales is vital to the industry’s financial stability. DDGS quality depends on maize grain quality differences on ethanol production efficiency during fermentation and also more likely to contain unsafe levels of fumonisins. The use of Bt hybrids should reduce detrimental health effects from fumonisins in DDGS fed to livestock. Impacts of grain quality differences on ethanol production efficiency during fermentation also are being investigated. Mulipurpose solutions (MPS) for disinfecting and storage of contact lenses often contain boric acid as a buffering agent. Limited published literature has suggested that boric acid and borate-buffered MPS may alter the corneal epithelium; an effect attributed to cytotoxicity induced by boric acid. However, this claim has not been substantiated and a thorough investigation of the ocular effects of boric acid and borate-buffered MPS is not currently available in the literature. We investigated the effect of treating cells with boric acid using the agar diffusion and elution assays, and also assessed the impact of boric acid on corneal epithelial barrier function. Boric acid was also assessed as an in vivo ocular model when administered for 28 days. Additionally, we evaluated an investigational boric acid-based MPS alone and with contact lenses for ocular compatibility in vitro and in vivo. Boric acid passed two different cytotoxicity assays (n = 3/assay). Boric acid did not alter the integrity of corneal epithelial tight junctions in vitro when tested at concentrations similar to in-use conditions (n = 3). Furthermore, boric acid was well-tolerated on eye follow-up repeated administrations in a rabbit model (n = 14). Finally, the investigational borate-buffered MPS demonstrated good ocular biocompatibility both in vitro and in vivo. This MPS was not cytotoxic by two assays (n = 3/assay) and was compatible with the eye alone and when evaluated with contact lenses. In summary, we provide evidence that boric acid and a borate-buffered MPS are compatible with the ocular environment by several different measures. Our findings provide evidence that ocular effects reported for some borate-buffered MPS are incorrectly attributed to boric acid and are more likely a function of additional components of the specific MPS formulation tested. The investigational MPS has not been reviewed by the US FDA and no determination of safety or effectiveness of the solution has been made by the agency.

**1168 NONCLINICAL SAFETY EVALUATION OF BORIC ACID AND A BORATE-BUFFERED CONTACT LENS CARE MULTIPURPOSE SOLUTION.**


The aim of the study was to evaluate subchronic, reproductive, developmental, and in vivo cytogenetic effects of Type III BURA fume condensate (RAFC) in Wistar (WU) rats. Reproductive/developmental toxicity was identified as the only data gap for asphalt under the U.S. High Production Volume (HPV) chemical program. RAFC was obtained from storage tanks at 201 °C and was shown to be representative to fumes collected at actual roofing operations. Animals were exposed to RAFC at target concentrations of 30, 100, and 300 mg/m3 THC (total hydrocarbons) or clean air by nose only inhalation, 6 hours per day, 7 days per week for at least four weeks (OECD 422). No adverse effects were seen on spermatology, behavioral endpoints or on any of the parameters of reproduction included postnatal development of F1 at all concentrations. RAFC did not induce any significant increases in micronucleus frequency in polychromatic erythrocytes of the bone marrow. No effects were seen on blood formation. Minimal adverse histopathological effects were observed in the lungs following exposure to 300 mg/m3 THC, consisting of a slight increase in alveolar macrophage accumulation, minimal mononuclear/inflammatory cell infiltration and minimal to slight (adaptive) alveolar hyperplasia of the bronchiolar type (alveolar bronchiolization). In all other organs, including larynx and trachea, no effects of the RAFC exposure were observed. The NOAEL for reproductive/developmental toxicity was 300 mg/m3 and 30 mg/m3 was determined to be the overall NOAEL for the study, with clear (mild) lung effects in the high dose and a significantly increased relative lung weight in the mid dose subchronic females although there were no histopathological correlates. The study was sponsored by the American Petroleum Institute.
GENOTOXICITY STUDIES ON REFERENCE SMOKELESS TOBACCO PRODUCTS USING GREENSCREEN HC GADD45A-GFP ASSAY.

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Some health experts have recommended that smokers, who refuse to quit or refuse to use nicotine replacement therapies, switch to low TSNA smokeless tobacco products (STP). Usual moist snuff is the most popular of the STP but has attracted much criticism from those against STP use on account of toxicological and addiction concerns. Use of standard in vitro assays (e.g., Ames) to assess STP toxicity was of limited utility in distinguishing product types and brands within a type (Rickert et al., Regul. Toxicol. Pharmacol. 2009 53:121-33). This study sought to assess the genotoxicity of DMSO extracts of the Reference Smokeless Tobacco products (151, 251, loose-leaf chewing tobacco; 152, dry snuff; 153, 253, moist snuff) in the increasingly widely used in vitro mammalian GreenScreen HC assay (GADD45a-GFP reporter host in TK6 cells). Extracts of tobacco products are inherently autofluorescent and so GreenScreen HC data were collected from flow cytometry to circumvent fluorescence interference. Genotoxicity data generated in this study for the five standard samples were not consistent with the levels of TSNA reported in these products (e.g., at 3h-S9 potency by LEC showed low TSNA STP activity was of limited utility in distinguishing product types and brands within a type, and blinded, and examined by fluorescence microscope. Over 2000 polychromatic erythrocytes on each slide were scored for the presence of micronuclei. Micronuclei frequencies were tabulated and statistical significance was determined by t test. A significant increase in micronucleus frequency, compared to vehicle controls, was induced by a 1mM, 1-hour exposure of 2,5-DMF with and without human liver S9 fraction metabolism. Further studies on the potential genotoxic effects of 2,5-DMF are underway.

REPEAT DOSE ORAL AND REPRODUCTIVE TOXICITY OF THE CHLORINATED FLAME RETARDANT DECHLORANE PLUS (DP).

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DP was introduced as a flame retardant as an alternative to brominated flame retardants but the published data are limited. Rats were treated with doses of 750, 1500 or 5000 mg/kg by gavage. Controls were treated with corn oil. For the subchronic toxicity phase, animals were observed for clinical signs of toxicity including FOB changes, body weight and food consumption changes and for effects on clinical and anatomic pathology. No effects were observed on clinical signs, body weights, food consumption, neurobehavioral and FOB evaluations. In addition, no effects were observed on clinical pathology parameters, and no organ weight effects were observed. In the reproductive toxicity phase, no effects were noted on clinical signs of toxicity, body weights or food consumption. Estrous cyclicity, reproduction and fertility indices, parturition (gestation length, litter size), pup body weights, sex ratios and clinical findings through LD4 were unaffected. No effects were noted on GD 20 uterine implantation data, fetal body weights or fetal sex ratios. No fetal external and visceral malformations or variations were observed. Mortalities occurred across all dose groups, including controls. Microscopic evidence of gavage-related errors consisted of adhesions, inflammation and fibrosis in the thoracic and pleural cavities with evidence of esophageal perforations noted in some animals. Microscopic findings associated with an antigens stimulus, immune response and/or a physiological stress response secondary to the presence of test article material in the thoracic cavity were observed. These findings were not considered to be test article related as they were not dose dependent and were observed only in animals with evidence of suspected gavage injury. Viscosity measurements of the DP suspensions in corn oil suggested that the high viscosity of the suspensions most likely contributed to the mis-dosing and mortalities observed in this study. The NOAEL was 5000 mg/kg.

EMODIN INHIBITED ATP BINDING TO TOPOISOMERASE II TO INDUCE DNA DOUBLE-STRAND BREAKS.

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Emodin has been widely used as a component of laxatives, whereas it can lead to genotoxicity. However, the mechanisms underlying its genotoxicity is not entirely clear. We applied different assays to investigate the mechanisms by which emodin induces genotoxicity. Without metabolic activation, emodin at 80 μg/mL was mildly genotoxic as indicated in thymidine kinase (TK) gene mutation assay and micronucleus (MN) test. But in the neutral comet assay and the detection of γ-H2AX, emodin at 80 μg/mL inhibited DNA double-strand breaks (DSBs). Moreover, results obtained from inhibitions of LDNA deconcatenation and relaxation of supercoiled pBR322 induced by topoisomerase II (Topo II) showed that emodin inhibited Topo II activity. Further, using both aclarubicin, a Topo II catalytic inhibitor, and HL-60/MX2 cells deficient in Topo II (Topo II) we showed that emodin-activated DSBs were in a Topo II--dependent manner. However, emodin did not intercalate into DNA. In contrast, emodin interacted with Topo II by competing with ATP for binding to the ATPase domain of human Topo II α and inhibited ATP hydrolysis. Taken together, these results suggested that emodin inhibited ATP binding to Topo II and induced DSBs.
Objectives: To determine the benefit of different rinsing protocols compared with no decontamination on an innovative ex vivo model. Methods: 86 explants human skin in 4 groups: 1 control and 3 exposed to hydrofluoric acid (HF) for 20 seconds by topical route from filter paper disks saturated with 30% skin in 4 groups: 1 control and 3 exposed to hydrofluoric acid (HF) for 20 seconds by topical route from filter paper disks saturated with 30% skin.

Results: Alternations searched for in stratum corneum, basal epidermis, papillary and reticular dermis. Control group: no lesions at any time. HF-exposed explants without decontamination: severe burns in the 4 layers, from 10 minutes onwards. Decontamination with tap water plus CaGlu: alterations of the 4 layers after 15 minutes, decreasing after 30 minutes. Recovery of lesions in epidermal cells from the 4th hour onwards and in dermal cells at 24h. Decontamination with Hexaflorine®, no epidermal or dermal cell alterations, even after 24h. These results are in accordance with those obtained on an ex vivo model for the eye. The effectiveness of Hexaflorine® decontamination, in this study, can be linked with successful results (without secondary care or systemic effects) obtained in 10% HF workplace splashes.

Conclusion: This new experiment helps to compare decontamination methods of 70% HF burns. Results are similar to those obtained in cases reports. Decontamination with tap water followed by CaGlu validates requirement of several and deeply penetrating applications of CaGlu to improve results. The effectiveness of using Hexaflorine® prior to any other protocol is confirmed.

**SAFETY OF GINGER OIL AND GINGER OLEORESIN AS FOOD INGREDIENTS.**

L. C. Dolan and G. Burdock, Burdock Group, Orlando, FL.

Ginger (Zingiber officinale Roscoe, family Zingiberaceae), a large, tuberous, perennial plant cultivated in many subtropical and tropical countries, is widely used throughout the world as a spice and has a long history of use as a medicinal herb (particularly for stomach upset). Ginger oil (the volatile oil obtained by steam distillation of dried ground rhizomes) and ginger oleoresin (obtained by hydrocarbon extraction of the dried, unpeeled rhizome) are used as flavor ingredients in foods and beverages. Ginger oil (1–3% of the rhizome) contains a complex mixture of terpenoids, high in sesquiterpene hydrocarbons, monoterpene and oxygenated compounds. The oleoresin contains 20–30% volatile oil, 10% “fixed” or non-volatile heavy oils, fats and waxes, and 50–70% pungent resinous constituents such as gingerol, shogaol, and paradol. The FDA has approved ginger oil and ginger oleoresin (solvent-free) as GRAS for use as flavoring agents and adjuvants in human or animal feed with no restrictions on food categories. Based on FEMA reported disappearance data, ginger oil consumption is 0.48314 mg/day or 0.008055 mg/kg bw/day and ginger oleoresin consumption is 1.7791 mg/day or 0.029661 mg/kg bw/day. The acute oral LD50 values of ginger oil and an ethanolic ginger extract (80%) (analogous to ginger oleoresin) are >5 g/kg bw in rats and >3.5 g/kg bw in mice, respectively. In humans, 4% ginger oil in petrolatum is not irritating or sensitizing. In vitro, ginger oil or ginger oleoresin inhibits growth of bacteria and fungi. Both materials exhibit mutagenic activity; however, they also suppress the activity of known mutagens. Pregnant rats administered up to 1000 mg/kg bw/day of a patented ginger extract (analogous to ginger oleoresin) orally from Days 6–15 of gestation, there were no adverse effects on the maternal animal or fetus. Although ginger oil and ginger oleoresin possess mutagenic activity and have not been tested for subchronic toxicity, consumption of these substances as ingredients added to food is considered safe at present use levels as demonstrated by a long history of safe use.

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Vegetative insecticidal proteins (Vip) represent a novel class of insecticidal proteins that are produced during the vegetative growth phase of the bacterium Bacillus thuringiensis. Vip3Aa2o, a variant protein sharing 99.7% amino acid identity with the native Bt Vip3Aa1 protein, is produced by event MIR162 genetically modified (GM) maize for the biological control of lepidopteran pests and is regarded as safe for consumption by humans and animals. A study was conducted to determine whether the Vip3Aa2o protein would exhibit a cytoxic effect on mamalian cells. Caco-2 (human epithelial cell line) and 3T3 (mouse fibroblast cell line) cells were independently exposed to various concentrations of Vip3Aa2o for 1 hour or 24 hours. Cytotoxicity was assessed using two different assays. The lactate dehydrogenase (LDH) assay measures the release of LDH from the permeabilized membranes of necrotic cells, while the neutral red (NR) assay measures the uptake of NR, which requires metabolic activity. The positive control, sodium lauryl sulfate, demonstrated the adequacy of the experimental conditions to control a cytotoxic response (increased LDH release or decreased NR uptake relative to control). LDH release for Vip3Aa2o treated cells ranged from 97-107% of control while the positive control increased LDH release to 158-186% of control. NR uptake for Vip3Aa2o treated cells ranged from 89-117% of control, while the positive control decreased NR uptake to 50-64% of control. There was no evidence of cytotoxicity resulting from exposure to concentrations of Vip3Aa2o up to 10,000 ng/mL in either cell line. An exposure concentration of 10,000 ng/mL represents a margin of exposure many thousands of times higher than would be expected as a result of even the highest dietary intake of GM maize. These data provide further evidence that the toxicological mode of action of Vip3Aa2o is highly selective for insects and not mammals.

**SAFETY ASSESSMENT OF THE INSECTICIDAL PROTEIN eCry3.1Ab, PRESENT IN GENETICALLY ENGINEERED SYN-O5307 MAIZE PLANTS.**


Genetically engineered SYN-O5307 maize plants produce eCry3.1Ab protein. The Bacillus thuringiensis derived eCry3.1Ab protein is a chimera of modified Cry3A and CryIAb proteins and has insecticidal activity against corn rootworm and related coleopteran species. As part of a safety assessment, bioinformatics homology searches for Cry3A and other Cry proteins were conducted to determine if the translated eCry3.1Ab protein has any unique features such as allergens, in vitro digestibility and in vitro toxicity studies were conducted. There was no significant sequence similarity between eCry3.1Ab and any known or putative toxic or allergenic proteins. eCry3.1Ab was readily digested after incubation in simulated gastric fluid containing pepsin. As assessed by SDS-PAGE and western blot analysis, test substance containing eCry3.1Ab was administered as a single oral dose via gavage to 5 groups of male and 5 female Fischer 344 rats at 0, 200, 2000, 20,000 and 200,000 mg/kg. Significantly increased body weight gain was observed at 2000 mg/kg; body weight increased approximately 8% at 20000 mg/kg and 15% at 200,000 mg/kg. Test substance containing eCry3.1Ab was administered to 10 Sprague-Dawley rats (5 per sex) and 8 Fisher 344 rats (4 per sex) at 0, 100, 500 and 1000 mg/kg/day via gavage on 5 days/week for 14 days. No test substance-related effects on mortality, clinical signs, body weight or nutritional parameters, and pathological changes at any dose. Statistically significant increases in liver weights at all dose levels (except 100 mg/kg/day males) and thyroid and kidney weights in 500 and 1000 mg/kg/day males were noted, but there were no associated histopathological or clinical pathology changes. The changes noted in the thyroid (altered mineralization; retention of basophilic material) and femur (increased mineralization) in all treated groups were not associated with clinical signs or microscopic changes and were likely related to free fluoride formed from 6-2 FMA metabolism. Plasma (3- to 4-fold) and urine (30- to 50-fold) fluoride was higher in treated groups versus controls. Therefore, the changes noted in organ weights and teeth or femur were not considered adverse. In the repeated dose toxicity study, the NOAEL was 1000 mg/kg/day. Overall, 6-2 FMA is considered to have low toxicity potential.

**PREPARATION AND STABILITY TESTING OF BUTYLPARaben FORMULATIONS IN RODENT FEED.**

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Butylparaben (n-butyl-p-hydroxybenzoate), a widely used preservative, has been selected for testing on the National Toxicology Program due to reports of estrogenic activity and adverse effects on the reproductive system in male rodents. Formulations were prepared in NIH-07 and NTP 2000 feeds at 1000 ppm. Samples of the formulations were extracted by rotating them end over end in acetone and analyzed by HPLC with UV detection. The predilution formulations were within 10% of the target concentrations. The formulations were stored at -20°C, 5°C, and room temperature, sampled, and reanalyzed at 1-week intervals. After 22 days of storage, significant declines (approximately 25% in the NIH-07 and 15% in the NTP-2000 feeds) in butylparaben concentrations were seen in the samples stored at room temperature. No degradant peaks were seen in the chromatograms. Binding was suspected since degradation of a food preservative would not be expected in feed. New formulations were prepared in both feeds at 4000 ppm to minimize the effects of binding and the stability study was repeated using a stronger extraction method (Sohxlet using acetonitrile for five cycles). Over 42 days of storage, a decline in concentration of approximately 50% was seen in the NIH-07 feed stored at room temperature. There was no significant decline observed in the NTP-2000 feed. Samples of the NIH-07 formulation stored at room temperature for 45 days were extracted directly in boiling acetonitrile (neutral, with added acid, and with added base). All three extracts had concentrations within 5% of Day 0, indicating the decline in concentration was due to binding rather than degradation. Butylparaben is known to bind to albumin and NIH-07 feed has a much greater protein concentration than the NTP-2000, which may account for the greater binding seen in the NIH-07. This work was supported by NTP Contract N01-ES-55551.
The intrathecal (IT) route of administration may be required for preclinical efficacy or safety assessments of agents to mimic the clinical regimen or to overcome toxicities which could be elicited upon systemic administration of neuroactive agents at levels that may be required to permit the desired IT exposure. Our laboratory has established surgical and technical procedures required for successful administration into the IT space either via injection or infusion in three common laboratory animal species: the albino rat, the beagle dog and the cynomolgus monkey. IT injections are performed under anesthesia as a direct puncture between the L4/L5 vertebrae (adults) or in consciously restrained animals (rat, dog and monkey) via a surgically implanted cannula connected to a subcutaneous access port. IT infusions utilize surgical implantation of a silicone-based catheter in the lumbar region between L2/L4 vertebrae for dogs and primates or at the cisternal level in rats. Injections or infusions of physiological saline into the IT space for 1 to 3 months using these procedures resulted in anticipated experimental-related histopathological changes. There were no effects on clinical condition, body weights, food consumption, or neurological effects associated with the experimental procedures. Clinical pathology parameters were judged to be slightly different from non-treated animals, considered likely due to the initial surgical implantation and/or the presence of a foreign material (catheter) in the IT space. Microscopic changes noted to the IT injections/infusions consisted of minimal hemorrhages and inflammation at the injection/infusion site. Compression of the spinal cord and degeneration of the nerve fiber can be occasionally associated with IT infusion. It can be concluded that in our laboratory, IT injections and/or infusions can be successfully performed in the albino rat, the beagle dog and the cynomolgus monkey with minimal experimental-related effects on clinical pathology, gross and microscopic examinations. The blood-brain barrier may hinder the delivery of potential therapeutic agents to the brain. In an effort to bypass this protective barrier, our laboratory previously validated intrathecal injection and infusion in rats as routes of cerebrospinal drug delivery. The cisterna magna (or cerebellomedullary cistern) is one port of entry to the spinal canal. Our laboratory evaluated, optimized and validated single injection/infusion procedures in the dosing period and were not considered primary toxic effects. In the 90-day study, no primary toxic effects on mortality, clinical signs, body weight parameters were observed at 1000 mg/kg/day only. Developmental toxicity was not observed at any dose. The NOAEL for maternal toxicity was 500 mg/kg/day, and for developmental toxicity was 1000 mg/kg/day, the highest level tested. No primary toxic effects on mortality, clinical signs, neurobehavioral assessment, body weight, nutritional parameters or clinical pathology (except granular casts in urine) were observed at any dose in the 90-day study. At ≥ 500 mg/kg/day, hyaline droplets accumulation associated with increased incidence and severity of chronic progressive nephropathy and granular casts was present in the kidneys of males. Degenerative and inflammatory changes associated with test material were present in the nose of animals from all groups, including controls. These changes were attributed to a dosing error, with secondary regurgitation, early in the dosing period and were not considered primary toxic effects. In the 90-day study, the NOAEL was 100 mg/kg/day for males based on species and sex specific kidney effects and 1000 mg/kg/day for females. In summary, the test substance is considered to have low human toxicity potential.
rats following 90-day oral (neurotoxicity) or 28-day inhalation (immunotoxicity) exposures. In the neurotoxicity study, ethylbenzene was administered orally via gavage twice daily at 0, 25, 125, or 250 mg/kg per dose (total daily dosages of 0, 50, 250, or 500 mg/kg body weight/day (mg/kg bwt/day)) for 13 weeks. This study included blinded systematic evaluation of functional and behavioral endpoints in an open field using a functional observational battery (FOB), automated assessment of motor activity including temporal pattern of change within a session, and pathologic examination of the central and peripheral nervous system following perfusion fixation. In the immunotoxicity study, animals were exposed by inhalation to 0, 25, 100, or 500 ppm ethylbenzene (approximately 26, 90, or 342 mg/kg bwt/day as calculated from physiologically-based pharmacokinetic modeling). Immunotoxicity was evaluated in female rats using the splenic Antibody-Forming Cell plaque-forming assay in deep red blood cell sensitized animals. The no-observed-effect level for the oral gavage study was 50 mg/kg bwt/day based on increased relative weights of the liver and kidneys in the male rats. The no-observed-adverse-effect level (NOAEL) for adult neurotoxicity was the highest dose tested, 500 mg/kg bwt/day. The NOAEL for the immunotoxicity evaluation was the highest tested exposure concentration, 500 ppm (342 mg/kg bwt/day).

192 HYDROFLUORIC ACID (HF) BURNS: A NEW EFFICACIOUS MODEL WITH EX VIVO BIO-EC HUMAN SKIN EXPLANTS.

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Objective: Hydrofluoric acid’s (HF) very hazardous properties are due to a double mechanism of action: corrosivity (H+) plus local and systemic toxicity (F-). A new efficacious skin model will allow a better understanding of burn mechanisms and in the future a comparison of first aid treatments. Methods: 59 human skin explants from abdominoplasty preserved in BIO-ECs Explant Medium at 37°C in a moist atmosphere with 5% CO2. HF exposure: 20 seconds by topical route from filter paper disks (9 mm in diameter) previously saturated with 30 μl of HF 70 %. Control group: no exposure. Histological sampling: at different times, from 1 minute up to 24 hours. Observation by optical microscopy X40. Results: Alterations, during penetration of HF were searched for in stratum corneum, basal epidermis, papillary and reticular dermis. The experiment made it possible to observe the progression of the lesions throughout the skin. After: 1 minute, beginning of penetration in the upper epidermis: 2 minutes, lesions reached epidermis basal layer; 3 minutes, epidermis was totally altered and first lesions appeared in the superficial part of dermis; 4 minutes, clearer alteration of papillary dermis; 5 minutes, alterations reached slightly reticular dermis. Beyond ten minutes, all four layers presented significant alterations. These lesions remain stable until the final observation after 24 hours when total epidermal necrosis can be observed. Conclusion: Under these operating conditions the kinetics of 70 % HF burns can be precisely analyzed. This model completely corresponds to the clinical lesions observed during accidental splashes. The direct effect of the corrosive agent is extremely rapid and the lesions progress very quickly. This study confirms the need for urgent and effective decontamination to prevent or minimize the severity of chemical burns due to concentrated hydrofluoric acid.

193 ERYTHROCYTOXICITY OF METHACRYLONITRILE IN MALHE SPRAGUE-DAWLEY RATS.

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Methacrylonitrile [MeAN, CH2=C(C3H3)CN] is a widely used industrial monomer in the production of plastic elastomers and coatings. It is also used in the preparation of acids, amides, amines, esters and nitriles. MeAN has been shown to be highly toxic in mice, rats and rabbits by dermal, respiratory and oral routes. It has also been shown to liberate cyanide ions in the blood of rats, mice and rabbits and its effects were diminished by standard therapy for cyanide poisoning. We have studied the effect of MeAN on erythrocytes of male Sprague-Dawley rats. Groups of rats (five each) were treated with 0.25 LD50 dose of MeAN dissolved in 0.2 ml safflower oil in one single oral dose. The control rats received only 0.2 ml safflower oil. The rats were observed for the visible signs of MeAN toxicity and were sacrificed in groups of five at 0, 1, 2 and 7 days after the MeAN treatment. The hematocrit, erythrocyte count, hemoglobin content and binding of MeAN to globin molecules were determined. The hematocrit of rats treated with MeAN showed a decrease of 15-20 % on day 1 and 2 which returned back to normal in a week. Erythrocyte counts were reduced to 70-75 % of control rats on day 1 and continued to be lowered even after 7 days. Similarly, the hemoglobin levels of rat treated with MeAN were shown to be less than 80 % of control rats. The highest binding of MeAN to globin molecules was found at day 1 and decreased significantly by day 7. Incubation of erythrocytes with MeAN renders their membranes susceptible to hypotonic lysis even at higher concentrations of NaCl as compared to normal erythrocytes. These data show that MeAN significantly affected the blood components of treated rats suggesting detrimental effect on functions of blood. [Supported by Grant No. S06RR08038 from the Minority Research Support Program of the National Institutes of Health]
Using RNAi high throughput technologies, we will identify chromatin proteins (including transcription factors, chromatin structural proteins, and regulatory proteins) that modulate the Aryl Hydrocarbon Receptor (AhR)-dependent induction of the CYP1A1 gene in the Hepa-1 murine hepatic cancer cell line. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and certain polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene are environmental contaminants and potent carcinogens. The mechanism of their toxicities lies in binding to the AhR. After ligand binding, AhR dimerizes with the Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) protein, and the AHRAHR dimer then activates transcription of CYP1A1, CYP1B1 and a number of other genes. Metabolism of PAHs by CYP1A1 plays a major role in carcinogenesis by this compound. TCDD is refractory to metabolism, but its carcinogenic effects also probably depend upon the activation of gene transcription by AhR/ARNT; although the responsible genes have not been fully defined. These cytochrome P450's then metabolize the PAHs to electrophilic derivatives that can mutate DNA. Further characterization of these processes is therefore a very important research objective. We have currently begun optimizing the production of endoribonuclease-prepared siRNAs (esiRNA) for use in this high throughput screening. In this assay, dsRNA (400-600bp in length) are produced, corresponding to specific genes, using a series of PCR steps and the addition of an AP primer. The barcoded enzyme RNase III is then used to cleave the dsRNA to approximately 21bp to generate esiRNAs efficient for transfection. Our esiRNA library of 1008 chromatin proteins will be used to transfect Hepa-1 cells, which will be treated with TCDD one day later, and after a further day assayed for CYP1A1 activity using the EROD assay. From these experiments, we expect to discover novel chromatin proteins involved in dioxin induced CYP1A1 expression.

Identification of Direct AhR-Regulated Genes Involved in B Cell Differentiation.

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent aryl hydrocarbon receptor (AhR) agonist, induces immunosuppression due in part to the altered differentiation of antibody secreting B cells. We have hypothesized that these alterations are due to a coordinated dysregulation of B cell gene expression mediated by AhR activation. To identify genes that are directly regulated by the AhR in differentiating B cells, the murine B cell line, CH12.LX, was exposed to lipopolysaccharide (LPS) to induce differentiation and AhR activation. To identify genes that are directly regulated by the AhR in differentiating B cells, the murine B cell line, CH12.LX, was exposed to lipopolysaccharide (LPS), an activator of B-cell differentiation, with and without TCDD treatment. Samples were collected at 1 hour post-treatment for whole genome chromatin immunoprecipitation analysis (ChIP-on-chip) and at 8 and 12 hrs for gene expression microarray analysis. The results identified 1893 genomic regions with a significant increase in AhR binding. Of these regions, 1035 mapped to within 10 kb of 803 genes. In the gene expression microarray analysis, 495 genes showed increased or decreased expression. A total of 78 genes showed both increased chromatin binding and altered expression. Among these several AhR-regulated transcription factors known to play a role in B-cell signaling and differentiation were identified including BTB and CNC homology 1, basic leucine zipper transcription factor 2 (BACH2). The ChIP-on-chip data was further analyzed for the enrichment of transcription factor binding sites. The results showed that additional transcription factors were responsible genes have not been fully defined. These cytochrome P450’s then metabolize the PAHs to electrophilic derivatives that can mutate DNA. Further characterization of these processes is therefore a very important research objective. We have currently begun optimizing the production of endoribonuclease-prepared siRNAs (esiRNA) for use in this high throughput screening. In this assay, dsRNA (400-600bp in length) are produced, corresponding to specific genes, using a series of PCR steps and the addition of an AP primer. The barcoded enzyme RNase III is then used to cleave the dsRNA to approximately 21bp to generate esiRNAs efficient for transfection. Our esiRNA library of 1008 chromatin proteins will be used to transfect Hepa-1 cells, which will be treated with TCDD one day later, and after a further day assayed for CYP1A1 activity using the EROD assay. From these experiments, we expect to discover novel chromatin proteins involved in dioxin induced CYP1A1 expression.

Hypoxia Inducible Factors Potentiate Aryl Hydrocarbon Receptor Signaling in Mesenchymal Stem Cells.

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Selective Suppression of Complement Expression by AhR.


The aryl hydrocarbon receptor (AhR) contributes towards pro and anti-inflammatory signaling through DNA-dependent and independent modes of action. Here we describe the AhR-mediated suppression of immune complement gene expression. Complement represents a potent non-specific surveillance mechanism to facilitate the elimination of stressed or damaged cells. However, chronic inflammation can lead to hyper-activation of complement with pathological consequences; hence attenuation of complement has obvious benefits. Human Huh7 cells express AhR and components of the complement system, which are induced by treatment with proinflammatory IL1β (2ng/ml). Huh7 cells exposed to the AhR ligand βNF (10µM) prior to IL1β displayed reduced expression of complement factors (C1R/C1S, C3, C5, CFH, CDS5) in conjunction with elevated phase I (CYP1A1) gene expression, as assessed by quantitative PCR. Stimulation of canonical DNA-dependent AhR-mediated phase I targets is potentially deleterious thus for AhR to be a viable therapeutic target selective AhR modulators (SARhMs), refractory to conventional AhR-dependent gene expression but retaining the capacity to stimulate repressive DNA-independent AhR activities are required. A screen identified the flavonoid 3’,4’-dimethoxy-4-naphthoflavone (DiMNF) as a candidate SARhM. DiMNF (10µM) was capable of diminishing complement gene targets in cytokine-stimulated Huh7 cells. Quantitative protein analyses revealed that diminished complement mRNA was reflected at the protein level. Interestingly, complement expression was attenuated by DiMNF without concomitant dioxin response element-driven phase I expression. Many flavonoids exhibit affinity for the AhR and competitive ligand binding assays confirm DiMNF as an AhR ligand with repressive activity. We suggest that the AhR represents a new target for the amelioration of specific aspects of inflammation e.g. complement; moreover identification and characterization of SARhMs, such as DiMNF may allow for the therapeutic application of AhR-dependent inhibition without conventional AhR ligand-mediated toxicities.

Genomic Analysis of Dioxin-Dependent Recruitment of AhR to Promoter Regions in Mouse Liver.

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Genome-wide, promoter-focused ChIP-chip analysis of hepatic AhR binding sites were examined in 8 week old female C57BL/6 mice that received a single i.p. injection of 30 µg/kg TCDD at 2 and 24 h post dose. Using a 1% false detection rate, 434 AhR-bound regions were in common between the two time points, corresponding to 403 unique genes. Conventional ChIP assays confirmed the ligand-dependent recruitment of AhR to many of the identified regions. However, significant ligand-independent binding to a select number of regions was also evident.
are essential for the proper folding and stability of the AHR, studies next evaluated observed when the yeast were treated with calpain inhibitors. Since MG132 also AHR is this yeast model. In addition, no reduction in AHR degradation could be implicated the 26S proteasome in the ligand-independent degradation of the blotting showed that treatment with MG132 blocked the degradation of the AHR. These results suggest i) that the ligand-independent degradation of the AHR in the recombinant yeast system is mediated by the 26S proteasome and ii) that the degradation is not the result of limiting levels of HSP90 expression. This model may be useful for future studies aimed at determining the mechanism of AHR degradation and the sites that are modified by ubiquitin. (ES015481)

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor with constitutive activities as well as those induced by xenobiotic ligands, such as the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). One unexplained cellular role for the AHR has been its ability to promote cell cycle progression in the absence of exogenous ligands, whereas treatment with exogenous ligands induces cell cycle arrest. Within the cell cycle, progression from G1 to S phase is controlled by sequential phosphorylation of the retinoblastoma protein (RB1) by cyclin D/CDK4/6 complexes. In this study, the functional interactions between the AHR, CDK4, and CCND1 were investigated as a potential mechanism for the cell cycle regulation by the AHR in human breast cancer cells. The results demonstrated that the AHR and CDK4 interact within the cell cycle and the interaction was disrupted upon TCDD treatment. The disruption was temporally correlated with G1 cell cycle arrest and decreased phosphorylation of RB1. Biochemical reconstitution assays using in vitro translated protein recapitulated the AHR and CDK4 interaction and showed that CCND1 was also part of the complex. In vitro assays for CDK4 kinase activity demonstrated that RB1 phosphorylation by the AHR-CDK4-CCND1 complex was reduced in the presence of TCDD. The results suggest that the AHR acts as a scaffolding protein and aids in the recruitment of RB1 to the AHR/CDK4/CCND1 complex. The recruitment of RB1 enables phosphorylation by CDK4 and facilitates cell cycle progression. Upon binding to an exogenous ligand, the AHR dissociates from CDK4 and CCND1 and the phosphorylation of RB1 is inhibited. This switch-like activity extends the reduced mammary development in AHR knockout mice and the inhibition of mammary tumorigenesis in the rodent bioassay.

The Aryl Hydrocarbon Receptor (AHR) is a ligand inducible cytosolic transcription factor that up- and down-regulates genes, resulting in a wide range of biological and toxicological responses. Although several studies have assessed AHR mRNA levels following exposure to polycyclic aromatic hydrocarbons and other AHR ligands, an antibody-based assay to quantify cytosolic AHR has yet to be established. Anti-AHR monoclonal antibodies to conserved peptide sequences at the N- and C-terminal of the AHR were used to capture AHR in hepatic cytosol fractions. The purified monoclonal antibodies have been shown to be reactive at levels as low as 5ng/ml when tested in an indirect enzyme immunoassay with the respective AHR peptide conjugates. Mouse Hepa-1 cells, cited to have the most abundant source of AHR, was used as one source of AHR. Cytosol preparations were also obtained from liver tissue samples of mice (C57BL/6 and BALB/c). AHR +/- mice, rat (Sprague Dawley) and human. Western blotting confirmed good species cross-reactivity of the monoclonal antibodies, with no signal present in cytosol from AHR +/- mice. The capture assay utilized either monoclonal anti-AHR developed to the N-terminal peptide, or monoclonal anti-AHR developed to the C-terminal peptide as the capture antibody. Next, application of cytosol preparations were followed by a detection system which consists of a goat anti-AHR and an alkaline phosphatase conjugated anti-goat secondary antibody. The capture assay gave a consistent linear detection system which consists of a goat anti-AHR and an alkaline phosphatase conjugated anti-goat secondary antibody. The capture assay gave a consistent linear signal with liver cytosol samples from mice, rat and human (0.3ng – 40μg total cytosol protein per well). The AHR monoclonal antibodies and capture assay for cytosol AHR will provide valuable tools for future studies on the function and regulation of the AHR. (supported by NIEHS grant RO3 ES012911)

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Previous studies have demonstrated that the aryl hydrocarbon receptor (AHR) is degraded following ligand binding. To generate a model system useful to screen for proteins involved in ligand-mediated AHR degradation, the cDNAs for mouse AHR and ARNT were stably integrated in the yeast genome. Initial studies have shown the AHR protein is rapidly degraded in a ligand-independent manner in these recombinant strains. The following project was designed in order to evaluate the mechanism behind this degradation event. The Egr6 cell wall protein was disrupted in the recombinant yeast strain to allow the cells to be permeable to various protease inhibitors. Recombinant yeast was grown in the presence of galactose in order to induce expression of AHR and ARNT. Following induction, cells were incubated in the presence of MG132 or Me2SO. Protein was extracted and western blotting showed that treatment with MG132 blocked the degradation of the AHR. This implicates the 26S proteasome in the ligand-independent degradation of the AHR is this yeast model. In addition, no reduction in AHR degradation could be observed when the yeast were treated with caplain inhibitors. Since MG132 also cause an induction in expression of heat shock proteins (HSP) and these proteins are essential for the proper folding and stability of the AHR, studies next evaluated whether the MG132 prevented AHR degradation as a result of increased HSP90 expression. To address this question, a constitutive Hsp90 expression plasmid was transformed into yeast and the stability of the AHR directly evaluated by western blotting. The overexpression of mammalian HSP90 did not significantly stabilize the AHR protein. These results suggest i) that the ligand-independent degradation of the AHR in the recombinant yeast system is mediated by the 26S proteasome and ii) that the degradation is not the result of limiting levels of HSP90 expression. This model may be useful for future studies aimed at determining the mechanism of AHR degradation and the sites that are modified by ubiquitin. (ES015481)
The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor capable of modulating gene expression by binding to a well-characterized xenobiotic response element (XRE), in response to agonists such as tetrachlorodibenzo-p-dioxin (TCDD). However, DNA microarray experiments performed in several laboratories including ours, identified numerous AhR target genes lacking an obvious XRE in the promoter or flanking regulatory regions. Our examination of one such gene, encoding plasminogen activator inhibitor type 1, identified and characterized a new non-consensus XRE (NC-XRE) that binds the AhR independently of the canonical AhR protein dimerization partner. Luciferase reporter expression studies and electrophoretic mobility shift assays confirmed AhR involvement and defined key nucleotides within the NC-XRE necessary for binding and function. Choromatin immunoprecipitation and shRNA-mediated AhR protein knock-down studies demonstrated that AhR-mediated activity through the NC-XRE does not require the AhR protein. Collectively, the evidence suggests that the NC-XRE represents a novel AhR binding site that defines a subset of target genes responsive to TCDD, distinct from the classical AhR/AhR-mediated genes. Supported by the NEIHS grants ES007800 and ES012018.

**1204 TCDD INDUCED PERICARDIAL EDEMA AND RELATIVE COX-2 EXPRESSION IN MEDAKA EMBRYOS.**

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Dioxin and other AhR ligands are well known to result in multiple defined developmental phenotypes including pericardial edema and circulation failure in small aquarium fish models. While these phenotypes are well described, the mechanistic underpinning behind these toxicities mainly remain elusive. To date, much of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity is thought to be due to the classical genomic actions of AhR/ARNT transcription factor complex. Here we suggest that AhR additionally functions through a non-classical, non-genomic mechanism(s) involving activation of inflammation and eicosanoid pathways. We demonstrate that medaka embryos exposed to TCDD (0.5-1 ppb) during early development result in a dose related increase in the prevalence of pericardial edema and that this phenotype correlates with modulation of both cyclooxygenase-2 (Cox-2) activity and gene expression in exposed medaka embryos. TCDD exposure significantly induces Cox-2 and CYP1A1 mRNA in all treated embryos. Those individuals exhibiting the edema phenotype were found to have significantly greater abundance of Cox-2 mRNA than the non-edema cohort. Biochemical inhibition of Cox-2 with NS-398 a selective Cox-2 inhibitor significantly attenuates the prevalence and severity of edema phenotype. Subsequently, exposures of medaka embryos to Arachidonic Acid (AA) resulted in recapitulation of the pericardial edema phenotype and significantly increased Cox-2 expression only in those individuals exhibiting the pericardial edema compared to the non-edema cohort. Interestingly, AA exposure does not result in any significant induction of CYP1A1 expression. Taken together, these results indicate a putative correlation between TCDD induced pericardial edema and relative Cox-2 mediated prostaglandin pathway in the medaka embryos. This work was supported by NCARS (02225), NCET-04-0262 and NSF (30360090).

**1205 A RAPID, CELL-BASED BIOLUMINESCENT ARYL HYDROCARBON RECEPTOR ACTIVATION ASSAY THAT USES CYP1A1 ENZYME ACTIVITY AS A SELECTIVE MARKER ACTIVITY.**


The aryl hydrocarbon nuclear receptor is activated for positive transcriptional regulation by small molecule drugs and toxins. This example can be used as a marker for AhR responsive gene. CYP1A1 expression can be measured at the levels of mRNA, protein expression and enzyme activity. Here we describe a rapid, non-destructive bioluminescent cell based assay for monitoring CYP1A1 enzyme activity as a marker of AhR activity. The assay uses a cell permeable bioluminescent CYP1A1 substrate. Bioluminescent substrates are probes that are converted by the enzymes of interest to a luciferin that is detected in a bioluminescent reaction with luciferase, correlating light output with target enzyme activity. The bioluminescent CYP1A1 substrate is a novel luciferin derivative that showed exquisite CYP1A1 selectivity over all other CYPs from a panel of 21 that were tested, including CYP1A2 and CYP1B1. The assay detected robust activations by known AhR agonists in a 96 well format that left cells intact for further analysis. While induction data with this assay correlated well with conventional mRNA and protein analysis methods, the luminescent assays were simpler, more rapid and by leaving cells intact allowed for a multiplexed assay that measured cell viability and AhR activity from single culture well. This bioluminescent assay enables a high throughput cell-based method for screening of AhR agonists.

**1206 SYNERGY BETWEEN ARYL HYDROCARBON RECEPTOR AND CONSTITUTIVE ANDROSTANE RECEPTOR FOR ACTIVATION PROMOTES MURINE LIVER HYPERPLASIA.**

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Mechanisms of hepatocyte proliferation triggered by tissue loss are distinguishable from those that promote proliferation in the intact liver in response to mitogens. Previous studies demonstrate that exogenous activation of the aryl hydrocarbon receptor (AhR), a soluble ligand-activated transcription factor in the basic helix-loop-helix family of proteins, suppresses compensatory liver regeneration elicited by surgical partial hepatectomy (PH). The goal of the present study was to determine how AhR activation modulates hepatocyte cell cycle progression in the intact liver following PH. We compared the effects of PH on the activity of the tumor suppressor p53 and CAR transcript and activity, which coincided with elevated levels of CAR protein and cyclin D1 expression. In contrast to the suppression effects of AhR activation observed during compensatory regeneration, TCDD pretreatment resulted in a 30-50% increase in hepatocyte proliferation in the intact liver of TCPOBOP-treated mice. Pretreatment with TCDD suppressed CDK2 kinase activity and increased the association of CDK2 with negative regulatory proteins p21Cip1 and p27Kip1, yet increased CDK4/cyclin D1 association and CDK4 activity, which culminated in enhanced phosphorylation of retinoblastoma protein. Moreover, pretreatment with TCDD transiently increased levels of CAR transcript and activity, which coincided with elevated levels of CAR protein and cyclin D1 expression. Based on these findings, we conclude that AhR activation potentiates TCPOBOP-stimulated hepatocyte proliferation through a mechanism that may include increased CDK kinase activity as well as elevated CAR expression and activity. Supported by the NEIHS Grant ES007800 (C. E.), F32ES013588 (K. M.), and F30ES016490 (S. W.).

**1207 TELOMERASE INHIBITION BY ARYL HYDROCARBON RECEPTOR (AhR) AGONISTS IN HUMAN MAMMARY EPITHELIAL CANCER CELLS.**

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Persistent organic pollutants (POP) are ubiquitous lipophilic chemicals. POP exposure has been suggested as a risk factor for breast cancer development based on some epidemiological and rodents studies. Other data suggest, however, that AhR agonists can cause anticarcinogenic effects. Telomerase expression is a key pro-carcinogenic component responsible for increased cancer cell longevity. This work aimed to test the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other AhR agonists on cell proliferation and telomerase expression in human epithelial mammary cancer cells. TCDD (5.3 nM, 6 d) decreased cell proliferation in two AhR-positive mammary cell lines, the estrogen receptor (ER)-positive T47D (36 % lower) and ER negative MDA-MB-231 (26 % lower), whereas the MDA-MB-231 cell line is resistant to TCDD. Similar results were observed with mixtures of PCBs, PCDFs and PCDDs. TCDD exposure of the AhR-negative neuronal cell line, SH-SYSY (ER-), did not affect cell proliferation rate nor telomerase activity. These data suggest an AhR-dependent suppression of telomerase expression by AhR agonists may represent a therapeutic pathway for breast cancer treatment.
INHIBITION OF ARYL HYDROCARBON RECEPTOR-DEPENDENT TRANSCRIPTION BY RESVERATROL OR KAEMPEROL IS INDEPENDENT OF ESTROGEN RECEPTOR ALPHA IN T-47D HUMAN BREAST CANCER CELLS.

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Resveratrol (RES) and kaempferol (KAE) modulate a number of different pathways acting as aryl hydrocarbon receptor (AhR) antagonists, but also as estrogen receptor alpha (ERα) agonists. Since ERα is present in the transcriptionally active AhR complex at AhR target genes and that there are controversial reports regarding the ability of ERα to modulate AhR signalling, we examined the role of ERα in RES- or KAE-mediated inhibition of AhR-dependent transcription. We observed that 10 μM of KAE efficiently antagonized dioxin-induced cytochrome P450 1A1 (CYP1A1) and CYP1B1 gene expression which compared to RES. Time course studies demonstrate that co-treatment with either RES or KAE inhibited CYP1A1 and CYP1B1 expression as early as 1.5 h after treatment. Both compounds effectively inhibited dioxin-dependent increase in CYP1A1 and CYP1B1 expression even after an initial 2 h pre-treatment with dioxin followed by 6 h co-treatment with RES or KAE. Chromatin immunoprecipitation assay reveal that the reduced CYP1A1 and CYP1B1 mRNA expression was due to reduced recruitment of AHR, ARNT and co-activators to the enhancer and promoter regions of these genes. Both compounds also induced recruitment of ERα to the ERα target gene, GREB1, at a level similar to that of E2. The TCDD-induced ERα recruitment to CYP1A1 and CYP1B1 was also inhibited by both compounds and RNAi-mediated knockdown of ERα had no effect on the ability of RES or KAE to repress AhR-dependent transcription. These data suggest that the estrogenic action and ERα do not contribute to the AhR-inhibitory properties of RES and KAE.

LIGAND SPECIFIC DIFFERENCES IN AH R DEPENDENT CHANGES IN HISTONE MODIFICATION.

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Dioxin and dioxin like compounds (DLCs) are environmental contaminants released during many industrial processes, particularly incomplete fossil fuel combustion. Halogenated and polyyclic aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), DLCs, and benzo[a]pyrene B[a]P are known or suspected human carcinogens and developmental teratogens and induce expression of the cytochrome P450 encoded by the subtype responsive CYP1A1 gene. CYP1A1 gene induction requires trans-activation by the heterodimeric transcriptional complex formed by ligand bound Aromatic Hydrocarbon Receptor (AhR) and the AHR Nuclear Translocator proteins (ARNT). We have shown that with B[a]P induced activation of Cyp1a1 gene expression in mouse hepatoma Hepa-1 cells, there is a concomitant dissociation of HDAC1 from the Cyp1a1 promoter and modification of the histone marks. Here we find that in mouse embryonic fibroblast, treatment with B[a]P shows a similar increase in Cyp1a1 transcription as in Hepa-1 cells accompanied by trimethylation of Lys4 of Histone H3 (H3K4) and hyperacetylation of H3K14. This strongly implies that this mechanism of response to aromatic hydrocarbons is common to many cell types. Because coplanar PCBs are AhR ligands and have toxic effects similar to TCDD we used chromatin immunoprecipitation to determine if TCDD and the coplanar PCBs cause a similar fingerprint of epigenetic modification as B[a]P. We found that all the AhR ligand treatments resulted in a similar epigenetic fingerprint, including hyperacetylation of H3K14 and H4K16, trimethylation H3K4 and phosphorylation of H3S10. Treatment with equivalent TEQ doses of PCBs results in increases in Cyp1a1 transcription, but the PCBs did not recruit AhR to the Cyp1a1 promoter in a manner consistent with their TEFs and showed a different pattern of histone modifications, suggesting that TCDD and the DLCs may have different mechanisms of action due to differences in epigenetic regulation.(Supported by ES06273, ES010807, T32ES016664-02)
permeability (calcine), mitochondrial membrane potential (TMRR), lysosomal integrity (rhodamine-dextran [RhDex]), chelatable iron (calcine), reactive oxygen species (ROS, cmH2DCFDA) and cell viability (propidium iodide [PI]).

RESULTS: KCN+IAA caused cell death, which was decreased by EDHB. Protection was associated with a delay of the lysosomal disintegration and mitochondrial depolarization that preceded plasma membrane failure and cell death. Cold-loading/warm incubation was used to load calcine into mitochondria and lysosomes. After KCN+IAA, mitochondrial calcine fluorescence became quenched, whereas lysosomal fluorescence increased, indicating lysosomal release of iron and its uptake into mitochondria. EDHB delayed these events. EDHB also blocked increased mitochondrial ROS formation after KCN+IAA. However, cell killing and EDHB cytotoxic protection against KCN+IAA were not altered by anoxia. Conclusions: EDHB protects against KCN+IAA-induced cell death. Protection is associated with prevention of iron translocation from lysosomes into mitochondria and a decrease of mitochondrial ROS. However, ROS under these conditions may not originate from molecular oxygen.
1218 INTRACELLULAR BAX TRAFFICKING: A DETERMINANT OF CELL DEATH?

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Brain damage due to neonatal hypoxia-ischemia (HI) is a major cause of morbidity and mortality in infants. Our long-term goal is to characterize HI-induced intracellular localization of Bax in order to develop intervention strategies that may prevent permanent damage in the developing brain of infants. Here we measured Bax multi-organelle localization after in vivo HI-injury and after 100% O2 resuscitation and its correlation with organelle-specific cell death signaling. In addition to examining HI- and HHI-treated cortical Bax protein, we used rotonone-treated P5 neuronal cortical cultures, differentiated PC12 and SY5Y cells to better characterize the role of Bax shuttling in cell death signaling cascades. In particular, we examined the role of Bax phosphorylation after apoptotic and necrotic-like cell death stimuli. We asked whether HI-dependent differential phosphorylation of Thr167 and Ser155 residues determines Bax intracellular localization. Neonatal (P7) brain HI induces intracellular translocation of Bax to the nucleus, mitochondria, and ER, where it triggers activation of cell death signaling cascades. When compared to HI-treated rat pups, we found that 100% O2 resuscitation of HI-treated (HHI) rat pups increases HI-induced ER Bax levels, ER-mediated cell death signaling, and lesion volume likely due to an increase in necrotic-like cell death, triggering an over-activation of inflammatory signaling. We also observed an increase in p-BaxThr167 in the nucleus of cortical tissue at 1 hour after HI when compared to sham. This suggests a role for phosphorylation in the translocation of Bax to the nucleus. Understanding the mechanisms of Bax translocation will aid in the rational design of specific therapeutic strategies which could potentially involve altering Bax subcellular localization to decrease the irreversible trauma resulting from a prolonged inflammatory response. Supported in part by NIEHS training grant award T32 ES007254; NCI/NCIHD grant HD093883 and grant #8590 from the Shriner's Children's Hospital.

1219 QUERCETIN INDUCES TUMOR-SELECTIVE APOPTOSIS THROUGH DOWN-REGULATION OF MCL-1 AND ACTIVATION OF BAX.

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Quercetin is a promising anti-tumor agent that induces apoptosis in a variety of tumor cells. Here we report that administration of quercetin in human leukemia cells resulted in a marked release of cytochrome c and smac/diablo, cleavage/activation of caspase-9, 7, 3, and apoptosis. Pronounced apoptosis was also observed in blasts from patients with different leukemia types but not in normal blood peripheral mononuclear cells. These events were accompanied by myeloid cell leukemia-1 (Mcl-1) down-regulation, most likely through a translational mechanism, in association with diminished cFLIP phosphorylation. Ecotopic expression of Mcl-1 but not cFLIP, reduced quercetin-induced cytochrome c release and cell death. In contrast, interruption of Mcl-1 by siRNA enhanced quercetin's ability to stimulate quercetin's lethality. We also found that in response to quercetin, Bax protein underwent conformational changes and then translocation to mitochondrial membrane which caused release of cytochrome c. Treatment with Bax siRNA resulted in a reduction of quercetin-induced apoptosis. Bax-negative human prostate cancer DU-145 cells were not sensitive to quercetin treatment and knockdown of Bax completely abrogated the activation of caspase 3, cytochrome c release and apoptosis. Data also show that Bax activation was mediated by caspase 8-dependent Bid cleavage and also by Mcl-1 down-expression. However the inhibi-
local caspase activation, caspase-activity and cleavage of inhibitor of caspase-acti- vated DNase (ICAD) were not inhibited whereas the extent of apoptotic chromatin condensation and DNA-fragmentation was decreased. Using the arylhydro- carbon receptor (AhR) antagonist CH-223191, the inhibiting effect of TCDD towards apoptotic chromatin condensation and fragmentation could be linked to AhR activation. Next, we investigated whether TCDD directly or indirectly influ- enced the activity of exogenous caspase-activated DNase (CAD). TCDD did not affect CAD-activity towards naked genomic DNA. Neither was CAD-activity modulated in nuclei isolated and treated with TCDD for 24 h. Furthermore, ac- tivity of CAD towards nuclei from cells irradiated with UV-light and treated subse- quently with TCDD for 1 h was not influenced. It appears plausible that TCDD inhibits apoptotic DNA-cleavage by some unknown mechanism in order to retain genomic integrity, allowing the cell to deal with xenobiotics despite an initiation of apoptosis. These cells could recover from an apoptotic insult, harbouring genetic aberrations. This could explain the tumor promoting potential of TCDD and might be an underlying mechanism for other tumor-promoters which induce xenobiotic metabolism.

**1223 LOCALIZATION OF ENDONUCLEASE G AND FRAGMENTED DNA DURING ACETAMINOPHEN LIVER INJURY IN MICE.**

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Acetaminophen (APAP) is the most common cause of acute liver failure in the United States. The morphological damage by APAP observed in the liver is cent- trilobular necrosis. It is associated with DNA fragmentation mainly by endonuclease G (EndoG) as determined by nuclear TUNEL assay, which is a surrogate method for apoptosis detection. Leakage of TUNEL-positive DNA fragments into the cytoplasm (cytoplasmic TUNEL) has been observed in some studies and inter- preted as a sign of necrosis. In the present study, we localized EndoG by immunos- taining and tested whether quantification of nuclear and cytoplasmic TUNEL may be used as a universal assay for APAP-induced liver cell damage that would allow sim- ultaneous detection of apoptosis and necrosis in the same cell. Mice were injected with APAP (300 mg/kg IP) and liver samples were taken at varying periods of time within 12 hours. There was no significant induction of EndoG during this time pe- riod, while some nuclear translocation of EndoG was observed. The treatment re- sulted in the increase of TUNEL-positive cells around the central veins between 2 and 12 hours after APAP injections. At the 2-hour time point, the TUNEL was mainly nuclear suggesting cell death by apoptosis. By 4 hours, TUNEL-positive material significantly leaked to cytoplasm, where it reached a maximum of 22% of total TUNEL, indicating the appearance of necrosis possibly associated with quick partial destruction of the nuclear membrane. At later time points, both nuclear and cytoplasmic TUNELs decreased suggesting leakage of plasma membrane due to further development of necrosis. These data provide evidence that apoptosis and necrosis coexist in liver during APAP injury, and that measurement of nuclear and cytoplasmic TUNEL may be a useful method for the assessment of apoptosis and necrosis coexisting in individual cells in vivo, and for measuring the progression of toxic liver injury.

**1224 ENDONUCLEASE G MEDIATES ENDOTHELIAL TOXICITY INDUCED BY CISPLATIN.**

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Cisplatin is one of the most frequently used drugs for the treatment of advanced breast cancer, lung cancer, testicular cancer and leukemia. It is known to induce end- othelial cell injury, which may lead to thrombotic complications and arterial hy- pertension, and significantly contribute to cardiac and kidney toxicities. Endothelial injury is a key mechanism in pathogenesis of the chemotherapy-induced vasogenic cerebral edema. The mechanisms of endothelial cell injury induced by cisplatin are unknown. Our recent studies strongly suggest that endonu- clease G (EndoG), a cytosolic mitochondrial enzyme, serves as a universal key molecule in caspase-independent apoptosis and autophagy in various in vitro and in vivo cell injuries induced by cisplatin. In the current study, we hypothesized that EndoG mediates cisplatin endothelial toxicity. We first demonstrated that cisplatin in therapeutic doses causes injury of human coronary artery endothelial cells (HCAECs) in vitro as determined by LDH release assay. EndoG expression meas- ured by cell ELISA showed that cisplatin-treated HCAECs express significantly more EndoG than untreated cells. Targeted inhibition of EndoG by siRNA led to above 70% protection of HCAECs treated with cisplatin (25 μM). To determine whether inactivation of EndoG is protective to endothelial cells in vivo, EndoG knock- out (KO) and control wild-type mice were subjected to single injection of cis- platin (6 mg/kg, IV). The number of rescued endothelial cells in aorta was evalu- ated using quantitative microscopy, while floating (dead) endothelial CD31-posi- tive endothelial cells in blood were quantified using flow cytometry. These experi- ments showed that endothelial cells in KO mice are significantly protected from cis- platin injury. Therefore our data suggest that EndoG plays causative role in cisplatin-induced endothelial toxicity and may potentially be used as a target for support therapy in order to prevent its cardiovascular complications.

**1225 1,1-BIS(3'-INDOLYL)-1-(P-SUBSTITUTED PHENYL) METHANES ACTIVATE MITOCHONDRIAL PERMEABILITY TRANSITION PORE-MEDIATED APOPTOSIS IN BOTH COLON AND PANCREATIC CANCER CELLS.**

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1,1-Bis(3'-indolyl)-1-(p-substituted phenyl)methanes (C-DIM) activate peroxisome proliferator-activated receptor gamma and grow hormone factor-induced Balbha (Nur77) and induce receptor-dependent and receptor-independent apoptosis in cancer cells and tumors. In this study, we investigated the activation of apoptosis in colon and pancreatic cancer cells by p-bromo substituted analogs (DIM-C-pBrH). DIM-C-pBrH activated the extrinsic and intrinsic apoptotic pathways and de- creased mitochondrial membrane potential (MMP) in both colon and pancreatic cancer cells. The mitochondrial permeability transition pore (MPTP) blocker cy- closporin A (CsA) inhibited DIM-C-pBrH -induced apoptosis and decrease of MMP indicating that DIM-C-pBrH-induced apoptosis is related to MPTP open- ing. CsA also activated the intrinsic apoptotic pathway, resulting in the induction of CCAAT/enhancer binding homologous protein, death receptor 5, and the extrinsic apoptotic pathway. Inhibitor studies showed that CsA, reactive oxygen species (ROS) inhibitor NAC and JNK inhibitor SP600125 blocked the JNK stress pathway in C-DIM-pBrH treated cells, indicating that C- DIM-pBrH activated JNK stress pathway is MPTP- and ROS-dependent. Further analysis showed that C-DIM-pBrH induced ROS generation which is also blocked by both CsA and NAC, indicating MPTP opening playing a role in C-DIM-pBrH induced ROS generation. Moreover, western blotting analysis showed that CsA in-hibited the activation of caspase-8 and caspase-9 while NAC only blocked the acti- vation of caspase-8. Thus, C-DIM-pBrH induced MPTP opening in both colon and pancreatic cancer cells, resulting in activation of the intrinsic apoptotic path- way and ROS generation. Subsequent activation of the JNK and the extrinsic apop- totic pathway was also dependent on opening of the MPTP complex and induction of ROS.

**1226 P62 SEQUESTERS KEAP1 INTO AUTOPHAGOSOMES, PREVENTING THE KEAP1-DEPENDENT UBQUITINATION AND DEGRADATION OF NRF2.**

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Protein degradation is tightly regulated by two major degradation machineries, the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP). Here, we report the cross-talk between these two systems through p62. We demon- strate that activation or inhibition of autophagy increases the formation of auto- phagosomes and thus, suppresses the degradation of a well-characterized UPS substrate, Nrf2, in a highly specific manner. Under basal conditions, Nrf2 is ubiqui- tinated by the Keap1-Cal3-E3 ubiquitin ligase complex and targeted to the 26S proteasome for degradation. In this current study, we show that upregulation of en- denogenous p62, or ectopic expression of p62, sequesters Keap1 into autophagosomes through direct interaction resulting in the inhibition of the Keap1-mediated Nrf2 ubiquitination and subsequent Nrf2 degradation by the UPS. In contrast, overex- pression of mutated p62, which loses its ability to interact with Keap1, had no ef- fect on Nrf2 stability. Moreover, overexpression of p62 had no effect on the stabil- ity of other UPS substrates, including IKBet, c-Jun, and cyclin A, B1, and D1, demonstrating its specificity for Nrf2. These findings contribute to our under- standing of how autophagy may regulate specific UPS substrates.

**1227 AUTOPHAGY: A KEY MECHANISM IN ARSENITE-INDUCED CYTOTOXICITY IN HUMAN LYMPHBLASTOID CELL LINES.**

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Arsenic is a ubiquitous environmental toxicant that is associated with a range of dis- eases and has a complex set of molecular targets in diverse tissue types, making it difficult to identify the mechanisms and pathways involved in arsenic-induced cy- totoxicity. One commonality in arsenic toxicity is its ability to induce apoptosis.
at a range of concentrations in a variety of tissue types. Despite this, alternative programmed cell pathways induced by arsenic have been proposed. Autophagy is involved in the degradation of damaged organelles and cellular proteins, and can also play a role in determining the fate of stressed cells. Human lymphoblastoid cell lines (LCL) have been used as in-vitro models in arsenic toxicology for many years, but the specific mechanism of arsenic-induced cytotoxicity in LCL is still unknown. We investigated the mechanism of sodium arsenite cytotoxicity in LCL 18564 using a combination of markers for cell death pathways. Apoptotic markers (phosphatidylserine externalization, PARP cleavage, and response to caspase inhibition) were all negative in arsenite-exposed cells. In contrast, electron microscopy images and acidic diphosphate fluorescence data indicated that autophagy is induced by arsenite and is correlated with arsenite-induced cytotoxicity in this LCL. It is unclear whether autophagy is a compensatory mechanism induced by arsenite exposure in LCL, or alternatively a cell death-effecting process in LCL. The ability of arsenite to modulate the autophagy pathway in LCL lends insight into the molecular mechanism of arsenite-induced cytotoxicity and suggests novel biologic targets in arsenic toxicology. (Funded by ES 00694, ES 16652, and ES 04940)

1228 RAPTOR, A COMPONENT OF MTORC1, PLAYS A KEY ROLE IN AUTOPHAGY IN ADAPTION OF RADIORESISTANCE IN A549 LUNG CANCER CELLS.
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Autophagy has been a sensational topic among oncologists recently, in that it plays a dual role – survival or death, depending on cellular circumstances. While incitement as well as mortality figures for lung cancer increase, the importance of controlling therapy-induced resistance rises accordingly. As radiotherapy-induced resistance in cancer cells has found to be mTOR-dependent, many approaches are made to enhance therapeutic efficacy by applying mTOR inhibitors to make the target cells more radioresilient. mTORC1, consists of Raptor, mLST, PRAS40, is an upstream negative regulator of autophagy, which works in a complex, however, the role of each components has not yet been clarified.

To test the basic role of raptor in gamma-irradiated A549 cancer cells, raptor-stably-overexpressed (A549-Raptor) and raptor-stably-downregulated (A549-shRNA Raptor) cell line were established and compared. As lysosome is a final destination for autophagic/lysosomal protein degradation, effects of raptor expression towards autophagy was examined. Expression of proteins involved in the formation and maturation of autophagosomes (beclin-1, LC3, Atg5) or associated with autolysosomes and lysosomes (LAMP-1, cathepsin D), as well as downstream proteins of raptor (p70S6K, elf4E) were determined. Interestingly after 72 hours of gamma-irradiation p70S6K was totally degraded in A549, but no change was shown in A549-Raptor, indicating that raptor might have an effect in radioresistance. Further observation has been made with treatment of chloroquine, the lysosomal membrane destabilizer – autophagy inhibitor, and increase of beclin-1 and procathepsin D, but not mature cathepsin D, and a decrease in expression of LAMP-1 were noticed. Taken together, control of raptor expression in cancer cells can be suggested to be a destabilizer – autophagy inhibitor, and increase of beclin-1 and procathepsin D, as well as downstream proteins of raptor (p70S6K, elf4E).

1229 MECHANISMS OF AMIODARONE AND DESETHYLAMIODARONE CYTOTOXICITY IN HUMAN LUNG CELLS.
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Amiodarone (AM) is a potent antidysrhythm agent which can cause potentially life-threatening pulmonary fibrosis, and desethylamiodarone (DEA) is a metabolite of AM that may contribute to the toxicity of AM in vivo. Recent evidence has implicated the involvement of the renin-angiotensin system (RAS) in the initiation and progression of amiodarone-induced pulmonary toxicity (AIFT). In HPLA1 human peripheral lung epithelial cells, we found AM to be converted to DEA minimally (<2%) after 24 h of incubation, indicating that the HPLA1 cell culture model can be used to observe the effects of AM and DEA independently. Apoptotic cell death was assessed by annexin-V-FITC and TUNEL, while necrotic cell death was determined by propidium iodide staining. The percentage of necrotic cells increased over six-fold after 24 h treatment with 20 µM AM (80.8%) compared to control (12.0%), while the percentage of apoptotic cells decreased from 8.26% (control) to 1.56% (p<0.05). In contrast, incubation with 5.5 µM DEA for 24 h increased the percentage of necrotic cells two-fold, from 10.8% (control) to 20.4%, and increased the percentage of apoptotic cells from 9.86% (control) to 22.0% (p<0.05). As determined by TUNEL, increased apoptosis was detected after 24 h treatment with 5.0 µM DEA (26.7%) compared to control (4.2%). Treatment with angiotensin II (100 nM – 1 µM) alone or in combination with AM or DEA did not alter cytotoxicity. Furthermore, pretreatment with the angiotensin converting enzyme inhibitor captopril did not protect against AM or DEA cytotoxicity. In conclusion, AM activates primarily necrotic pathways, whereas DEA activates both necrotic and apoptotic pathways, and the RAS does not seem to be involved in AM or DEA cytotoxicity in HPLA1 cells. Multiple mechanisms may contribute to the initiation of lung damage observed clinically, due to actions of both AM and its metabolite DEA. (Funded by CIHR Grant No. MOP-13257).

1230 EVALUATION OF MOTOR ACTIVITY AND BRAIN MORPHOMETRICS IN DNT STUDIES WITH OPS.
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Developmental neurotoxicity (DNT) studies have been submitted to EPA’s Office of Pesticide Programs for 18 organophosphate (OP) pesticides for purposes of pesticide registration under the Food Quality Protection Act. DNT studies, conducted in rats using a standardized test guideline, include detailed neurobehavioral and neuropathological assessments, following in utero and postnatal exposures. In this analytic, motor activity data and brain morphometric data from these 18 studies were extracted and analyzed. These endpoints were selected from the DNT studies as they have been shown in the literature to be affected by OPs, particularly chlorpyrifos. Motor activity data (means and standard deviation at each block) from rats PND 60+ were plotted as box and whisker plots. Some data from OPs analyzed showed increases in variance. However, variability of size and orientation of sectioning as well as in parameter measured restricted comparison across the 18 OPs. In addition, small sample size makes interpretation and statistical analysis challenging.

1231 PARAQUAT INDUCES BOTH NIGRAL AND STRIATAL DOPAMINERGIC DAMAGE IN OCT3-/- MICE.
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We previously reported that the organic cation transporter-3 (Oct3) played a critical role in MPTP and methamphetamine neurotoxicity by bi-directionally regulating the local bioavailability of toxic cation neurotoxictants (Cui et al, PNAS, 2009). Because paraquat is also a cation, we asked whether Oct3 also modulated neurodegeneration in the paraquat mouse model. To this end, we injected Oct3-/- and their littermates Oct3+/+ with paraquat (10mg/kg i.p. every 2 days for 10 injections). Consistent with the neurotoxic features of the paraquat model, we detected a loss in nigral dopaminergic neurons (~33%), but no striatal damage in Oct3+/+ mice. In contrast, we did observe a significant loss (~40%) in striatal dopaminergic terminals in Oct3-/- mice. The mechanism by which paraquat induced striatal toxicity in the absence of Oct3 function is unclear; however, upon a closer investigation of the transport kinetics of paraquat through OCT3, we discovered that although paraquat was a very poor substrate for OCT3 (Fig 7c, d) - despite the similar structure of this cation to MPP+, it did interact with OCT3, as evidenced by the inhibition of MPP+ uptake through this transporter. Taken together, our study suggests that Oct3 plays a significant role in paraquat toxicity and furthermore, Oct3-/- mice may represent a novel paraquat mouse model of Parkinson’s disease, in which both nigral and striatal damage occur.

1232 PYRETHROID INHIBITION OF A HUMAN T-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL IS STRUCTURAL SPECIFIC AND CONCENTRATION-DEPENDENT.
E. M. Mutanga, Z. H. Valentine and S. B. Symington. Biology and Biomedical Science, Salve Regina University, Newport, RI.

Pyrethroids are widely used insecticides in both agricultural and vector control programs. Given the widespread use of these compounds for the control of insect vectors of devastating human and animal diseases and additional exposure via dietary uptake, human consumption is virtually assured. The purpose of this research was to determine the effects of pyrethroid insecticides as a class on the current charac-
teristics of a human T-type voltage-sensitive calcium channel (Cav3.2). Human Cav3.2 cDNA was transcribed into cRNA, injected into defollicled Xenopus oocytes, and the resulting currents electrophysiologically characterized using a two-electrode voltage clamp technique with Ba2+ as a charge carrier. Concentration-dependent response curves were generated and relative indices of potency and efficacy obtained following perfusion with various concentrations of bifenixin, bioal- lethrin, α-cyhalothrin, β-cyfluthrin, deltamethrin, esfenvalerate, fenvalerate, fen- propithrin, permethrin, and tefluthrin. Overall, pyrethroids did not modify Cav3.2 in a consistent manner. Three different groups were identified by a cluster analysis of the relative indices of toxicity obtained from the concentration-dependent response curves. Group 1 pyrethroids consisted of permethrin and fen- propithrin and elicited no effect on Cav3.2. Group 2 pyrethroids consisted of bifen- thin, bioalithrin, and tefluthrin and were moderately potent and efficacious inhibitors of Cav3.2 peak current. Group 3 pyrethroids consisted of α-cyhalothrin, β-cyfluthrin, deltamethrin, esfenvalerate, and fenvalerate and were most potent and efficacious of the compounds analyzed. Thus, most α-cyano pyrethroids are potent and stereospecific inhibitors of the human Cav3.2, however, these compounds as a class, do not modulate Cav3.2 in a consistent manner.

**1235** Utility of C6-Glioma Cells for Exploratory Risk Assessment of Complex Mixtures of Organophosphate and Pyrethroid Insecticides.

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Real environments and food products may carry low levels of multiple hazardous compounds. According to recent toxicological information from rats and environmental data, organophosphate (OP) and pyrethroid (PYR) insecticide residues pollute the environment. In outdoor settings, each single exposure alters the low levels that may be considered safe for humans. Yet, there is a data gap on the neurotoxicology of complex insecticide mixtures. Here we present initial data of an exploratory, in vitro, pesticide toxicity model aimed to identify toxicologically relevant combinations of insecticides that require cumulative risk assessment efforts in in vivo models of greater human relevance. As a first task, we used rat C6-glioma cells to determine sub-effective dose-ranges for cell viability. Cell cultures were examined after insecticide exposures for 4, 12, 24 and 48 hr by optical and fluorescence microscopy. In addition, the MTT assay was carried out as a test for mitochondrial stress and general cell damage. Data from MTT assays were modeled applying Benchmark Dose Software (BMDS) analysis. Using the MTT test, we generated time- and dose (0.1 up to 50-250 μM)-effect data for two OP and two PYR compounds. In general, sub-μM exposures produced no clear evidence of cytotoxicity. Higher concentrations produced dose-related drops in cell viability at 24-48 hr, save the OP acephate. The BMD15s were 6.7 μM for Chlorpyrifos, 4.4 μM for Bifenithrin, and 44 μM for Tefluthrin. Moreover, a dose-related increase in cell death-related morphological events was evident using Hoechst 33258 histological evaluations from the low μM range up. We are presently evaluating other compounds and carrying out AChε assays using the cell-viability safe dose-range determined as mentioned.

**1236** Cellular Consequences of Dieldrin Analog Exposure in Dopaminergic Cells.

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Parkinson's Disease (PD) is a progressive disorder that leads to the degeneration of dopaminergic neurons in the substantia nigra. This neurodegeneration has been shown to significantly correlate with a number of environmental factors, including exposure to pesticides such as the organochlorine insecticide, dieldrin. This pesticide is ranked one of the twelve most persistent, bioaccumulative, and toxic chemicals by the U.S. EPA. Previous studies found an increased concentration of dieldrin in the striatal region of brains of PD patients, and that dieldrin adversely affects a variety of insecticides that require cumulative risk analysis efforts in in vivo models of greater human relevance. As a first task, we used rat C6-glioma cells to determine sub-effective dose-ranges for cell viability. Cell cultures were examined after insecticide exposures for 4, 12, 24 and 48 hr by optical and fluorescence microscopy. In addition, the MTT assay was carried out as a test for mitochondrial stress and general cell damage. Data from MTT assays were modeled applying Benchmark Dose Software (BMDS) analysis. Using the MTT test, we generated time- and dose (0.1 up to 50-250 μM)-effect data for two OP and two PYR compounds. In general, sub-μM exposures produced no clear evidence of cytotoxicity. Higher concentrations produced dose-related drops in cell viability at 24-48 hr, save the OP acephate. The BMD15s were 6.7 μM for Chlorpyrifos, 4.4 μM for Bifenithrin, and 44 μM for Tefluthrin. Moreover, a dose-related increase in cell death-related morphological events was evident using Hoechst 33258 histological evaluations from the low μM range up. We are presently evaluating other compounds and carrying out AChε assays using the cell-viability safe dose-range determined as mentioned.

**1234** Developmental Exposure to Deltamethrin Results in Gender Specific Changes in Hepatic UDP-Glucuronosyltransferase Expression.

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Developmental exposure to a commercial formulation of deltamethrin has been reported to induce several P450 isozymes in liver and brain (Johri et al., 2006), suggesting that exposures such as these may have an impact on future metabolism of endo-and xenobiotic substances. However, there is no information on the ability of developmental deltamethrin exposure to alter the expression of Phase II enzymes. One of the main families of Phase II enzymes are the uridine diphosphate-glucoro- nonsyltransferases (UGTs), which catalyze the addition of UDP-glucuronic acid to endogenous and exogenous compounds to facilitate their excretion. In this study, we sought to determine whether developmental deltamethrin exposure altered the expression of hepatic UGTs, focusing on the UGT1 and UGT2 families. Pregnant C57BL6 mice were administered vehicle (corn oil), 1 mg/kg, or 3 mg/kg deltamethrin every three days throughout gestation and lactation. The offspring were weaned at day 22 and sacrificed at 11 and 12 months of age. Livers were har vested and RNA isolated for QPCR. Developmental deltamethrin exposure resulted in increased expression of several UGT isoforms, but only in the male offspring. In the UGT1 family, UGT1A1 was increased by 54% and 119%, UGT1A2 was increased by 105% and 466%, UGT1A6 was increased by 31% and 90%, and UGT1A9 was increased by 32% and 149% in the low and high dose groups, respectively. In the UGT2 family, UGT2B35 was increased by 13% and 84% in the low and high dose groups, while UGT2B36 was increased by 22% in the high dose group. These data suggest that developmental exposure to deltamethrin causes long-term changes in UGT expression in a gender-related manner, which may influence the disposition and metabolism of endogenous and exogenous chemicals that are substrates for the UGTs. Supported in part by NIEHS Grant R01ES015991 and T32ES007148.

**1233** Early Differential Necrosis and Apoptosis Initiate and Contribute to the Development of OPIDN: A Study of Molecular, Cellular, and Anatomical Studies.

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Oxogonorphosphorus-ester induced delayed neurotoxicity (OPIDN) is a neurodegenerative disorder characterized by ataxia progressing to paralysis with a concomitant central and peripheral, distal, axonopathy. Dioxoarylphosphorofluoridate (DFP) produces OPIDN in the chicken that results in mild ataxia in 7-14 days and severe paralysis as the disease progresses with a single dose. White leghorn layers hens were treated with DFP (1.7 mg/kg sc) after prophylactic treatment with atorpin (1 mg/kg sc) in normal saline and eserine (1 mg/kg sc) in dimethyl sulfoxide. Control groups were treated with vehicle propylene glycol (0.1 ml/kg sc), atropine (1 mg/kg sc), atorpin (1 mg/kg sc) and eserin (1 mg/kg sc) in normal saline and eserine (1 mg/kg sc) and atorpin (1 mg/kg sc) in dimethyl sulfoxide. Data from three experiments were analyzed for mRNA (northern) and DNA (laddering) studies. Northern blots were probed with GAPDH, beta actin, 18S RNA to investigate their expression pattern. Data from MTT assays were modeled applying Benchmark Dose Software (BMDS) analysis. Using the MTT test, we generated time- and dose (0.1 up to 50-250 μM)-effect data for two OP and two PYR compounds. In general, sub-μM exposures produced no clear evidence of cytotoxicity. Higher concentrations produced dose-related drops in cell viability at 24-48 hr, save the OP acephate. The BMD15s were 6.7 μM for Chlorpyrifos, 4.4 μM for Bifenithrin, and 44 μM for Tefluthrin. Moreover, a dose-related increase in cell death-related morphological events was evident using Hoechst 33258 histological evaluations from the low μM range up. We are presently evaluating other compounds and carrying out AChε assays using the cell-viability safe dose-range determined as mentioned.
Chlorpyrifos (CPF), a widely used organophosphorus (OP) pesticide, is metabolized either to an inactive metabotrolo, tri-chloro-2-pyridyl (TCPy), or to chlorpyrifox oxon, which is a potent cholinesterase (ChE) inhibitor. Urinary TCPy levels have been shown to be a reliable biomarker for CPF exposure. TCPy inhibition activity is an indicator of CPF toxicity. Previous work has identified extensive neurobehavioral deficits in Egyptian agricultural personnel involved in pesticide application. In the present study, Egyptian agricultural workers (n=57) with job titles of pesticide applicator, technician or engineer, were studied during 9 to 17 consecutive days of CPF application to cotton fields in the summer of 2008. Urinary TCPy (daily) and blood butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) activities (every 7-8 days) were measured as biomarkers of CPF exposure and effect. Average TCPy levels for applicators, technicians and engineers were 6.089, 252, and 505 μg/g creatinine, respectively, with a range of 1.6 to 32.3. These are substantially lower than normal occupational exposures. To address this question, we examined effects on learning and memory in adult Long Evans rats exposed to chlorpyrifos (CPF) at levels approximating those observed in Egyptian pesticide workers applying CPF during the 2008 cotton season. Prior to exposure, food-restricted rats learned an appetitive Pavlovian discrimination between two tones, one that signaled food pellet delivery and one that did not. Exposure began on the final day of this training with rats injected subcutaneously with CPF at 0, 3 or 10 mg/kg/d over 20 days. For half of the rats, the contingencies were reversed during CPF exposure (reversal group); for the other half, the contingencies remained the same (maintenance group). Although CPF exposure appeared to subtly alter the maintenance and reversal of appetitive behavior, these effects were not reliable. These findings suggest that subchronic exposure to CPF at levels that do not cause systemic cholinergic toxicity do not significantly interfere with motivation for food, previously established performance, or the acquisition of a new discriminative response. Following the 20-day CPF exposure, rats received Pavlovian fear conditioning, a single-trial learning task in which a white noise stimulus was paired with a mild foot shock. In contrast to the appetitive learning task, CPF treatment caused robust dose-related deficits in cued and contextual conditioning. These findings suggest a role for amygdala and hippocampal function in CPF-associated behavioral impairments in rats. Supported by NIH grant #ES16308 (Anger and Lein, MPI).

Organophosphates are the most commonly used pesticides worldwide. Human and animal studies clearly identify neurotoxicity as the primary endpoint. However, determining whether repeated low-dose exposures to OPs pose a risk to humans has been difficult because: 1) a relationship between OP dose and behavioral deficits has not been defined in humans; 2) biomarkers that reliably predict OP-induced behavioral deficits from chronic exposures are lacking; and 3) the influence of genetic variation on exposure sensitivity is not known. We have designed coordinated human and rodent studies to better define the risks and mechanisms by which repeated low-dose OP exposure induces neurotoxicity. To test the hypothesis that chronic OP neurotoxicity is dose-related and that measures of oxidative stress and inflammation are better predictors of behavioral deficit than cholinesterase inhibition. The human studies focus on pesticide application workers in Egypt's Menoufia Governorate to: 1) estimate OP dose using PBPK/PD modeling of urinary OP metabolite data and data on genotype for polymorphisms of key enzymes involved in OP metabolism (CYP2B6, CYP2C19, PON1); and 2) determine the relationship between OP dose and neurobehavioral deficits. Our initial field studies in Egypt have identified significant dermal exposure (as high as 422ug/cm2/hr), elevated urinary metabolite levels and suppressed plasma cholinesterase in pesticide workers. PBPK/PD modeling using these data confirmed comparable doses in the rodent model we will use to test the effectiveness of urinary TCPy, cholinesterase inhibition and peripheral measures of oxidative stress and inflammation to predict behavioral deficits. Results of these studies will facilitate identification of at-risk individuals and testing intervention and treatment strategies (NIH ES16308).

Insecticides targeting acetylcholinesterase (AChE) are becoming less practical due to growing resistance among target insects as well as their toxicity to humans. This has led to researches inductive to agriculture and insects that are capable of serving as disease vectors. We have reported the finding of a free cysteine (Cys) residue at the entrance of the active site of AChE in some insects but not in mammals, birds, or fish. We also reported a Cys-targeting molecule, AMS18, that irreversibly inhibited 100% of AChE activity extracted from aphids, 95% activity extracted from the malaria vector mosquito (Anopheles gambiense s. str. larvae), carbamate fungicide that is used on a variety of plant diseases. It is also used as an accelerator in rubber manufacturing, packaging materials, and textiles. In spite of their generally acknowledged low toxicity, diisothiocarbamates are known to cause a wide range of neurobehavioral effects as well as neuropathological changes in the brain. Diisothiocarbamates are a family of highly reactive compounds due to their metal combining capacity and their ability to interact with sulphydryl-containing compounds. Acrystox play a key role in normal brain physiology and in the pathology of the nervous system. This investigation studied the effects of ziram on rat hippocampal astrocytes. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS. Acrystoxes were exposed to 1μM of ziram for one hour at 37 degrees C and then re-fed with complete media for 24 hours. The measurement of thiobarbituric acid reactive substances (TBARS) is a method for screening and monitoring lipid peroxidation. The TBARS assay performed showed a significant increase, p < 0.05, in malondialdehyde, a product of lipid peroxidation, in the ziram treated cells when compared to controls. Hsp70 is a stress protein whose expression is upregulated when the cell is placed under conditions of stress or exposure to toxic chemicals. Hsp70 decreases in concentration of this heat shock protein in the ziram treated cells as compared with control cells. This data suggests that the cytotoxic effects observed in the ziram treatments may be related to an increase of oxidative stress.

Organophosphorus pesticides (OPs) are the most commonly used pesticides worldwide. Human and animal studies clearly identify neurotoxicity as the primary end point of concern. However, determining whether repeated low-dose exposures to OPs pose a risk to humans has been difficult because: 1) a relationship between OP dose and behavioral deficits has not been defined in humans; 2) biomarkers that reliably predict OP-induced behavioral deficits from chronic exposures are lacking; and 3) the influence of genetic variation on exposure sensitivity is not known. We have designed coordinated human and rodent studies to better define the risks and mechanisms by which repeated low-dose OP exposure induces neurotoxicity. To test the hypothesis that chronic OP neurotoxicity is dose-related and that measures of oxidative stress and inflammation are better predictors of behavioral deficit than cholinesterase inhibition. The human studies focus on pesticide application workers in Egypt's Menoufia Governorate to: 1) estimate OP dose using PBPK/PD modeling of urinary OP metabolite data and data on genotype for polymorphisms of key enzymes involved in OP metabolism (CYP2B6, CYP2C19, PON1); and 2) determine the relationship between OP dose and neurobehavioral deficits. Our initial field studies in Egypt have identified significant dermal exposure (as high as 422ug/cm2/hr), elevated urinary metabolite levels and suppressed plasma cholinesterase in pesticide workers. PBPK/PD modeling using these data confirmed comparable doses in the rodent model we will use to test the effectiveness of urinary TCPy, cholinesterase inhibition and peripheral measures of oxidative stress and inflammation to predict behavioral deficits. Results of these studies will facilitate identification of at-risk individuals and testing intervention and treatment strategies (NIH ES16308).
>80% of the activity from the yellow fever mosquito (Aedes aegypti L.) and the northern house mosquito (Culex pipiens L) while an identical exposure caused no effect on human AChE. We have also reported the crystal structure of AChTS13 re- vealed bound to recombinant mouse AChE (mAChE). We now report 75% in- hibition of AChE activity extracted from cockroach (Blattella germanica and Periplaneta americana), >85% inhibition of beetle (Tribolium confusum Jacquelin du Val and Harmonia axyridis) AChE activity, and 90% inhibition of yellow jacket (Vespula maculifrons) AChE using AChTS17 while having no effect on human AChE activity under the same conditions. The experimental procedures used to complete this study included AChE assay based on an established radiometric tech- nique utilizing tritium-labelled substrate and time course studies of the irreversible inhibition of AChE. Our current study confirms the viability of Cys-targeting in- hibitors as insecticides for a broad range of insect pests and disease vectors.

1242 ACHÉ DEPRESSION IS RELATED TO OP METABOLITES IN URINE OF ORCHARD WORKERS PERFORMING THINNING.

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Exposure to organophosphate pesticides (OPs) is an occupational hazard for farm workers. We conducted in 2005 a longitudinal cohort study of farmworkers and non-farmworkers in the Yakima Valley of Washington State (WA). We are reporting the relationship of acetylcholinesterase (AChE) depression to the concentration of OP dialkyl metabolites during the thinning season, when farmworkers repeatedly sprayed orchards after a waiting period to thin trees. During the thinning season the dialkyl metabolite with the highest concentration was diethylphospho- late (DMP). For both farmworkers 63 μg/L 95% CI=(4,6) and non-farm- workers 5 μg/L 95% CI=(4,6) based on three urine collections two days apart. DMP having the highest concentration in urine probably is the result of the use of azinphos methyl, which is the most common OP used during the thinning season and is found in the highest concentration in dust from homes and vehicles of the farmworkers. Both RBC and plasma cholinesterase was measured using a field test kit and AChE depression was computed as the ratio of the thinning season (April to July) to the off season AChE values (November to February). We found a signifi- cant depression for RBC AChE with a regression coefficient of 0.12% depression per μg/L of DMP 95% CI=0.07,0.17). Most of the farmworkers had relatively low values of RBC AChE depression, 90% had less than 25% depression and only 2 of the 65 farmworkers had depression levels greater than 40%. This suggests that even farmworkers who perform tasks such as thinning which is only performed after spraying are still at risk for depression of AChE and that some fraction of these workers may have depression that exceeds 40%. (Supported by grants 2-P01-ES009601 from National Institutes of Environmental Health Sciences and RD-833454) (This abstract does not reflect EPA policy)

1243 INHIBITION OF ACETYLCHOLINESTERASE (AChE) AND BUTYRYLCHOLINESTERASE (BCHE) IN HUMAN BLOOD FOLLOWING IN VITRO AND IN VIVO EXPOSURE TO CHLORPYRIFOS.

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Chlorpyrifos (CPF), a widely used organophosphate (OP) pesticide, is metabo- lized to trichloro-2-pyridinol (TCPy), a CPF specific inactive metabolite, and to chlorpyrifos oxon, which is a potent cholinesterase (ChE) inhibitor. In vivo expo- sure to CPF was monitored in Egyptian cotton field workers (n=57) during CPF application by measuring urinary TCPy and blood AChE and BCHE levels as bio- markers of exposure and effect, respectively. A wide range of CPF exposures, based on urinary TCPy, was directly related to the inhibition of AChE and BCHE activi- ties as measured by the portable Test-mate ChE System. Following 3 to 9 days of CPF application, AChE was inhibited from 0 to 95%, while BCHE was inhibited from 0 to 100%. To assist in validation of estimates of CPF exposure and PBPK modeling in humans, an improved and automated in vitro kinetic assay based on the Ellman method was developed to measure AChE and BCHE in small quantities of human whole venous blood samples following in vitro exposure to CPF and CPF oxon. Exposure to CPF oxon over a range of final concentrations of 0 nM to 200 nM, yielded corresponding AChE and BuChE inhibition up to 100% for both en- zymes, enabling us to correspond specific concentrations of active metabolite to in vitro ChE inhibition. In vitro studies over a wide range of concentrations of both

1244 THE EFFECTS OF THE FUNGICIDE MANEB (MANGANOUS ETHYLENEDIIS(DITHIOCARBamate)) ON RAT HIPPOCAMPAL ASTROCYTES.

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Dithiocarbamates have been shown to induce reactive oxygen species (ROS), de- plete antioxidant levels and induce oxidative damage. The fungicide Maneb an eth- ylenediis(dithiocarbamate) has been shown to have high acute toxicity to aquatic life. The exposure of pesticides maybe also related to chronic disorders of the mam- malian CNS. This relationship between dithiocarbamates and neurodegenerative disorders is increasingly being studied. The purpose of this study is to investigate the effects of Maneb on the mammalian astrocyte. Rat hippocampal astrocytes were maintained in Dulbeco’s modified Eagle’s medium at 37°C. 80% CO2 and sup- plemented with 10% FBS. Cell viability for Maneb treated astrocytes was deter- mined using a trypan blue exclusion assay. The LC50 of Maneb, astro- cyttes were treated for 24 hours with various concentrations at 60-70% confluence. The LC50 value was calculated to be 13.6 μM. At the end of the treat- ment, cells were harvested for biochemical assays. The TBARS assay showed a sig- nificant increase (p<0.05) in MDA, a product of lipid peroxidation, in the Maneb treated cells when compared to controls. In contrast, a significant increase (p<0.05) in the antioxidant levels in Maneb treated cells was also observed. Morphological observations were performed using both phase-contrast and scanning electron micro- scropy. Treated astrocytes appeared rounded, vacuolated and contained dense bodies. Electron microscopy showed membrane blebbing, loss or retraction fibers and altered cell processes when compared to control cells.

1245 PYRETHROID INSECTICIDE ACCUMULATION IN PRIMARY CULTURES OF CORtical neurons IN VITRO.

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Primary cultures of neurons have been widely utilized to study the actions of pyrethroids and other neurotoxins, with the presumption that the media con- centration accurately reflects the dose received by the cells. However, recent studies have demonstrated that lipophilic compounds (e.g. methylmercury, PCBs and PBDEs) rapidly accumulate in cells to concentrations much higher than in the sur- rounding media. To test the hypothesis that pyrethroids rapidly accumulate in neu- rons in vitro, the time (0-90 min) and concentration (0.05 – 10 μM) dependence of accumulation of [14C]-deltamethrin (DM), [14C]-bifenthrin (BF) and [14C]-cy- clopyrol (CP) into primary cultures of cortical neurons was examined. Accumulation of all three pyrethroids was time- and concentration-dependent, with only small differences observed between the compounds. Concentration-de- pendent accumulation of PM and BF were similar, achieving a total -0.25 nM in cells after 30 min in a 10 μM solution. DM accumulation was lower, reaching a maximum of 0.14 nM after 30 min in a 10 μM solution. In 1 μM solutions, DM and PM content in cells was 0.039 and 0.038 nM after 90 min. At all concentra- tions and times, pyrethroid accumulation in cells was less than 3% of the total radioactivity applied. The amount of radioactivity recovered in the media at the end of incubation ranged from -75% - 98%; the remainder (0 to 25%) was presumed to bind to the plastic of the culture plates. These results demonstrate rapid time- and concentration-dependent accumulation of pyrethroids in neurons in vitro. Further, for the three pyrethroids examined, there do not appear to be large differ- ences in their accumulation. This data will be useful for making comparisons be- tween in vivo and in vitro studies regarding effective concentrations of pyrethroids. (This abstract does not reflect EPA policy)

1246 COMPARISON OF EEG CHANGES PRODUCED BY CARBARYL (CARBAMATE), PERMETHRIN (TYPE I PYRETHROID), AND DELTAMETHRIN (TYPE II PYRETHROID).

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We have reported that treatment with carbaryl may alter Theta activity in the EEG (Lyke et al., Toxicologist, 108S(1-1):441, 2009). In this study, we examined the abil- ity to detect changes in EEG activity produced by pesticides with different modes
of action. Long Evans rats were implanted with epidural screw electrodes. After recovery, non-restrained animals were gavaged with corn oil and tested for 2 days for acclimation. On day 3, the rats were dosed (po) with vehicle (corn oil), a carbamate (carbaryl, 50 mg/kg), a Type I (Bemethrin, 42 mg/kg), or a Type II pyrethroid (Deltamethrin, 2.5 mg/kg). The carbaryl-treated animals and their controls were tested 30 min later. The pyrethroid-treated animals and their controls were tested 2 h later. These times were associated with maximal inhibition of brain cholinesterase (carbaryl), or peak time of inhibition of motor activity for the pyrethroids (about an ED₅₀ dosage). EEG was recorded as 30 segments of 2 s, transformed using a FFT, and the spectra averaged. Because of the different time of maximal effect for the different classes of chemicals, the data were transformed as a percent of their respective control mean. Treatment with carbaryl reduced the peak frequency for the slower components of the EEG (Delta and Theta, 64-79% of control) compared to the pyrethroids (92-107% of control). Permethrin-treated animals had greater amplitude and/or area of multiple higher frequency regions of the EEG (Alpha, Beta, Gamma, 109-125% of control) compared to carbaryl (70-102% of control) and/or deltamethrin (82-98% of control). This was due to a combination of permethrin-induced increases and carbaryl-induced decreases in these measures. The results indicate that the non-stimulus driven EEG was differentially altered by pesticides with different modes of action.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

**1247 AGING-RELATED CARBARYL EFFECTS IN BROWN NORWAY RATS.**


The rapid increase in older adults in the population highlights the importance of understanding the role of aging in susceptibility to environmental contaminants. As part of a larger research program on life-stage susceptibility, this experiment determined the effect of the carbamate pesticide carbaryl on the locomotor activity of adolescent, young-adult, middle-age and senescent male Brown Norway rats. Locomotion was evaluated during 30-min sessions in photocell devices that recorded both horizontal and vertical activity. Carbaryl (17 mg/kg) and corn oil vehicle were administered p.o. at varying times (30, 60, 120 and 240 min) prior to a test session. The rats were 1 mo (n=18), 4 mo (n=18), 12 mo (n=18) and 24 mo (n=14) at the beginning of the experiment. Each rat received both corn oil and carbaryl at weekly intervals; treatment times were arranged in a mixed order. Following corn-oil treatment, horizontal activity was highest in 4-month rats then decreased at older ages. Vertical activity generally decreased with age. Except for the 240-min treatment time, carbaryl decreased both horizontal and vertical locomotor activity in all rats. Carbaryl effects were next expressed as a percentage of each rat's vehicle response then averaged across rats in each age group. Carbaryl produced proportionately greater effects in older rats. Older rats also took longer to recover following carbaryl treatment. The results demonstrate the importance of assessing toxic effects across a range of ages, from adolescence to senescence, in order to make informed estimates of risk. These results also indicate aging may increase susceptibility to environmental contaminants.

(This abstract does not necessarily reflect U.S. EPA policy.)

**1248 REPEATED EXPOSURE TO LOW DOSES OF CHLORPYRIFOS: HIPPOCAMAL DAMAGE, DEFICITS IN SPATIAL LEARNING AND INHIBITION OF THE ERK-CREB SIGNAL SYSTEM.**

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Chlorpyrifos (O,O’-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothionate, CPF) is one of the most widely used organophosphates (OPs) pesticides throughout the world. CPF is the commonly used insecticides for residential pest control in agriculture in China. Since the main target of OPs is acetylcholinesterase (AChE), which hydrolyses acetylcholine (ACh) in cholinergic synapses where this enzyme plays a key role in cell to cell communication. It is well known that the acute toxic effects of OPs are the accumulation of ACh in the synapses induces hyperactivity in cholinergic pathways. But there are more puzzles left within the low-level chronic exposure, especially the dosages without eliciting any systemic toxicity. The purpose of this study was, therefore, to evaluate the behavioral changes and signal systems involved of repeated, subthreshold doses of CPF in rats. In the present study, Male Wistar rats were given 0, 1.0, or 5.0mg/kg/day of CPF by intragastric administration daily for 4 weeks. The lowest dose bracket the threshold for barely-detectable cholinesterase inhibition, and have no effects on weight gain. Performance in a spatial learning task was impaired after 28 days of exposure to CPF, locomotor activity in the novel open field was changed, especially the centre place spending time increased. Although hippocampal morphology after CPF exposure was normal on gross observation, morphometric analysis revealed a significant overall reduction in the total number of neurons and significance thinning of the CA1 layers. The phosphorylated protein expression of ERK I/II and CREB decreased, but not the phosphorylated CaMKII. Collectively, these results indicate that repeated exposures to subthreshold doses of CPF may lead to hippocampal damage and behavioral abnormalities, and suggest the involvement of ERK-CREB signal system in the functional central nervous system alterations. Supported by China Natural Science Foundation Grant 30800929.

**1249 KINETIC OF THE ESTEREOSPECIFIC INTERACTION OF A MODEL CHIRAL PHOSPHORAMIDATE WITH ESTERASES IN PERIPHERAL NERVE SOLUBLE FRACTION.**


Toxicity and biotransformation of chiral organophosphorus compounds is depending of the stereospecificity of the interaction with esterases of their stereo-isomers. O-Hexyl, O,2,5-dichlorophenyl phosphoramide (HDCP) is a chiral compound synthesised as analogue of the commercial insecticide methamidophos (O,O-di-methyl phosphoramidothioate) for the study of the toxicological and biotransformation properties. Kinetic data were obtained for different R(+)-HDCP and S(-)-HDCP esterifications incubated for thirty minutes with soluble fraction of chicken peripheral nerve. A kinetic model equation was deduced assuming a multi enzymatic system with two different molecular phenomena occurring simultaneously: (1) Inhibition and (2) simultaneous spontaneous reactivation. The best fitting model is compatible with three sensitive enzymatic components (E1=47-49%, E2=42%, E3=10-11%) with both stereoisomers. The corresponding second order rate constants of inhibition (ki) were 0.0327, 0.0057 and 3.06x10⁻⁵ N-mₐ⁻₁min⁻¹, for R(+)-HDCP and 0.1387, 0.1401 and 4.4x10⁻⁵ N-mₐ⁻₁min⁻¹ for S(-)-HDCP. The kinetic of inhibition and the kinetic of reactivation (after inhibition and re-moving inhibitor) were consistent with significant spontaneous reactivation of E1 and E2 inhibited with R(+)-HDCP (kr=0.0068 and 0.0659 min⁻¹) but only E2 inhibited with S(-)-HDCP (kr=0.0116 min⁻¹). The interaction of the isomers of phosphoramidates (HDCP as a model) is different with the different esterase fraction in the sensitivity and the capacity of spontaneous reactivation.

**1250 ROLE OF CALCIUM INFUX AND CALPAIN ACTIVATION IN PYRETHROID PESTICIDE-INDUCED DOWN-REGULATION OF SODIUM CHANNEL EXPRESSION.**

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The voltage gated sodium channel (Na(v)) is responsible for the rising phase of the neuronal action potential and as such is a critical part of neuronal signaling. Previous in vitro studies have reported that incubation with sodium channel agonists results in the internalization and degradation of Na(v) alpha subunit protein and down-regulation of mRNA (Sharlazi et al., 2001). However, the mechanisms by which this long-term down-regulation of mRNA expression occurs are not clear. Furthermore, it has not been determined whether these effects occur in vivo. Recent work from our laboratory has determined that developmental exposure to the pyrethroid pesticide deltamethrin decreased Na(v) 1.3 expression by about 27% and Na(v) 1.1, Na(v) 1.2, and Na(v) 1.3 expression by about 24% in cortex and striatum, respectively. In order to better understand the mechanisms responsible for these persistent alterations of sodium channel expression we exposed human neuronal stem cells (SK-N-AS) to the pyrethroid pesticide deltamethrin (100 nM) and monitored mRNA expression of Na(v) 1.2 and Na(v) 1.3. Following 24 hr of exposure, Na(v) 1.2 and 1.3 mRNA was reduced by about 20% in the SK-N-AS cells. This effect was completely abolished by pre-treatment of the cells with the Na(v) blocker tetrodotoxin. These decreases were also blocked by depletion of intracellular calcium by BAPTA-AM, suggesting that calcium influx following the initial Na+ influx plays a key role in the decreased expression. Pre-treatment of cells with the proteasome inhibitor lactacystin and the calpain inhibitor PD-150666 also prevented the decrease in Na(v) mRNA expression. Because lactacystin has been demonstrated to reduce depolarization-induced calcium entry (Li et al., 2007) and calpain is activated by calcium, these data suggest that deltamethrin-induced depolarization and subsequent calcium influx activates calpain, which initiates degradation of Na(v) protein and mRNA. Supported by R01ES015991.
DEVELOPMENTAL EXPOSURE TO DELTAMETHRIN RESULTS IN GENDER SPECIFIC CHANGES IN CARBOXYLESTERASE EXPRESSION AND INCREASED METABOLISM OF METHYLPHENIDATE.

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Several in vitro studies have demonstrated that pyrethroid pesticides, including deltamethrin, induce P450 gene expression through activation of the nuclear receptor PXR. Activation of PXR has also been demonstrated to induce carboxylesterase (CES) expression in vivo. CES plays an important role in mediating the metabolism of pyrethroid pesticides and a number of therapeutic drugs including methylphenidate. Here, we sought to determine whether developmental deltamethrin exposure altered the expression of PXR and CES1 and whether the metabolism of methylphenidate was altered. Pregnant C57BL/6 mice were administered vehicle (corn oil) or 3 mg/kg deltamethrin every three days throughout gestation and lactation. The offspring were weaned at day 22 and sacrificed at 11 and 12 months of age. Livers were harvested and RNA isolated for QPCR. Developmental deltamethrin exposure induced CES1 expression by 126% in the male offspring, with no significant effect in the female offspring. The expression of PXR was not affected by developmental deltamethrin exposure. However, we did observe increased expression of the nuclear receptors CAR (66%) and RXRα (44%) in male offspring exposed to deltamethrin during development. To determine whether this induction of CES1 alters the metabolism of methylphenidate, mice were administered methylphenidate (4 mg/kg/p.o.). Brain levels of methylphenidate were decreased by 56% in male offspring developmentally exposed to deltamethrin. These data suggest that developmental exposure to deltamethrin causes long-term changes in CES1 expression that enhances the metabolism of methylphenidate. Furthermore, the increased CES1 expression appears to be the result of the induction of RXRα and CAR expression. Supported by NIH grant 1U01NS063723.

FULLERENE ANTIOXIDANTS DECREASE ORGANOPHOSPHATE-INDUCED ACETYLCHOLINESTERASE INHIBITION IN VITRO.

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Fullerene nanomaterials were examined as novel agents against organophosphate (OP)-induced acetylcholinesterase (AChE) inhibition using 2 in vitro test systems, hen brain and human neuroblastoma SH-SY5Y cells. Different derivatized fullerene structures (hydroxylated C60, carboxylated C60, C60-pyridoline, hydrosyoxylated C70, C70-bis malonal, hydroxylated scandium and gadolinium C80 Trimetaspheres™), were incubated with paraoxon (0 = control, 0.05, 0.1, 0.2 and 0.3 μM) or diisopropylphosphorofluoridate (DFP, 0 = control, 5, 10, 20, 50 μM) for 15 min before AChE assays. Activity of brain and SH-SY5Y AChE in the presence of these concentrations of OP compounds alone ranged from 17%–45% of control for paraoxon and from 8%–40% of control for DFP. Incubations containing 1 and 10 μM fullerenes were able to bring AChE activity of hen brain at least 20% closer to control when the two lowest concentrations of paraoxon and DFP were used. These changes were statistically significant. None were effective at meeting the criteria of 20% improvement in hen brain AChE activity at the highest concentrations of paraoxon and DFP. Effectiveness of the fullerenes against OP-induced AChE inhibition in neuroblastoma cells was mixed, but at least 4 of the fullerenes were effective at 10 μM against 0.01 μM paraoxon and 10 μM DFP. Using dissipation of supernate anion radicals as an indicator (sambamine oxidation as a positive control), all fullerenes demonstrated significant antioxidant capability in neuroblastoma cells at 1 μM concentrations. No fullerene at this concentration significantly affected neuroblastoma cell viability; when determined using either Alamar Blue or a luminescent assay for ATP production. These studies suggest that fullerene nanomaterials have potential capability to ameliorate OP-induced toxicities. Supported by NIH grant U10NS063723.

VESTIBULAR TOXICITY OF cis-CROTONONITRILE IN 129S1 FEMALE MICE PRE-TREATED WITH DIALLYLSULFIDE.

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Most small alkyl nitriles release cyanide by metabolism. Some, including 3,3’-iminodipropionitrile, allyl nitrite and cis-crotononitrile, cause degeneration of the auditory and vestibular hair cells after systemic administration in the mouse and other species. Recent data demonstrate that cis-crotononitrile is a CYP2E1 substrate, and that this CYP2E1-mediated metabolism associates with cyanide release and acute lethality, but not with vestibular toxicity. We hypothesized that pharmacological CYP2E1 inhibition could favor expression of vestibular toxicity while limiting cyanide-associated lethality. Female 129S1/SvImJ mice were exposed to cis-crotononitrile at doses of 0, 1.75, 2.25, 2.75, or 3.25 mmol/kg (po), in either a baseline condition or following exposure to the CYP2E1 inhibitor, diallylsulfide (200 mg/kg, po, 2 h before cis-crotononitrile). Vestibular toxicity was assessed using a specific behavioral test battery at 2, 7 and 21 days after exposure. Significant vestibular dysfunction was recorded in the groups of mice dosed with cis-crotononitrile at 2.25 mmol/kg or higher. This was not modified by diallylsulfide pre-treatment, that, is similar levels of vestibular dysfunction were recorded in the two groups of mice treated with the same cis-crotononitrile dose. In contrast, diallylsulfide caused a reduction in mortality, and significant differences in survival were recorded between pre-treatment groups after 2.75 mmol/kg of cis-crotononitrile. The vestibular toxicity of cis-crotononitrile in mice pre-treated with diallylsulfide may constitute an animal model useful in the study of mechanisms of ototoxicity as well as for the development of strategies for hair cell protection or repair. Work supported by the Ministry of Science and Innovation, Spain (BFU2006-00343/BFI).

NEUROTOXICANTS MALATHION AND LEAD ACETATE INCREASE GENE EXPRESSION OF SCAFFOLD PROTEINS ZO1 AND ZO2, AND CALCIUM CHANNEL PROTEIN TRPC1 IN ENDOTHELIAL CELLS.

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ZO1 and ZO2 are scaffold proteins that support tight junction structures in the blood-brain barrier (BBB). Damage to these proteins affects tight junction structure and therefore compromises BBB function. Transient receptor potential canonical channel protein 1 (TRPC1) forms store-operated calcium channels associated with the calcium signaling that regulates cell permeability in the endothelial cells of the BBB. Since lead is known to mimic calcium in several calcium related pathways, it may affect TRPC1 expression. The present experiments assessed changes in gene expression of ZO1 and ZO2 scaffold proteins, and of TRPC1 protein in rat brain vascular endothelial cells (RBE4) in response to treatments with neurotoxics malathion and lead acetate alone and in combination. We utilized real time polymerase chain reaction (Q-PCR) analysis to assess changes in patterns of gene expression for these proteins, applying the comparative CT method for relative quantification. Lead alone did not increase gene expression for any of the genes at any of the concentrations tested. In contrast, when applied alone, malathion increased gene expression for all three genes. Combinations of malathion and lead significantly increased gene expression of ZO1, ZO2, and TRPC1 proteins. Increases of ZO1 and ZO2 expression may be indicative of damage to the scaffold support of the tight junctions; increases in TRPC1 expression may be associated with formation of more store-operated calcium channels, which would increase intracellular accumulation of lead. In both events, the immediate result is the increasing permeability of the BBB.

ALTERATIONS IN MITOCHONDRIAL DYNAMICS AND AXONAL TRANSPORT AFTER EXPOSURE TO CHLORPYRIFOS AND CHLORPYRIFOS-OXON IN RAT CORTICAL NEURONS.

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The organophosphate chlorpyrifos (CPF) is one of the most commonly used agricultural pesticides in the US and it can persist in the soil, water, and food supply for months after application. We have previously reported that repeated low-level exposures to CPF (i.e., exposures not associated with signs of acute toxicity) result in progressive impairments in the performance of memory-related tasks in rodents as well as deficits in axonal transport in sciatic nerves ex vivo. Here we investigated the effects of CPF and its active metabolite CPFoxon on neuronal function at the mitochondrial level in vitro, specifically axonal transport of mitochondria and mitochondrial dynamics (i.e., processes critical for determining size, morphology and distribution of mitochondria). Alterations in mitochondrial transport and dynamics can result in the disruption of the energy supply in neurons and the maintenance of synapses potentially leading to neuronal dysfunction and neurodegeneration. We used primary cell culture (rat cortical neurons) to evaluate mitochondrial accumulation and morphology in axons after 1, 12 and 24 hour exposure to 5, 10
and 20uM CPF and CPFoxon. Neuronal imaging revealed an elongated morphol
(increase in length and decrease in number of mitochondria in axons) and
paired axonal transport of mitochondria that appeared to be dose dependent. These
changes were observed without signs of cell death or compromise of the mitochon
tral membrane potential. Our data suggest that exposure to relatively low levels of
CPF and its metabolite CPFoxon results in deficits in axonal transport and mito
chondrial hyperfusion leading to mitochrondial misplacement. Such alterations in
neuronal function may contribute to the CPF-related cognitive deficits we have
previously observed in rats.

1256 EFFECT OF LOW LEVEL EXPOSURE TO DISOPROPYLFLUOROPHOSPHATE (DFP) ON REGIONAL BRAIN METABOLISM IN F344 RATS.
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The mechanism of organophosphate (OP) induced cholinergic toxicity is a poten
tially catastrophic event that is well described. Exposure to OPs at noncholinergic
doses has been reported to cause aberrations in neuronal development and signa
deficits in cognitive behavior and spatial memory. However, little is known about
the mechanisms of action for noncholinergic toxicity. It is hypothesized that
low level exposure to OPs causes oxidative stress and alterations in brain metabo
lism. Using multinuclear NMR techniques the metabolic effects of DFP in rat
brain be investigated and correlated with oxidative stress and acetyl
cholinesterase (AChE) inhibition. Adult male Fischer 344 rats were administered
1 mg/kg DFP or saline via subcutaneous injection. Animals were euthanized at 0.5,
1, 2, 12, 24 and 48 hr post dose. Brains were removed and cortex, cerebellum, hip
cocampus and brainstem were collected. Lipid and aqueous extracts were prepared
from each brain region, and profiles of small molecule metabolites, lipids and phos
pholipids were measured using 1H, 13C and 31P NMR spectroscopy. Oxidative
stress was measured using malondialdehyde (MDA). All brain regions reached a
minimum of AChE activity (40-55%) at 1-2 hr post dose with the exception of cor
tex which had a minimum of activity at 12 hr post dose. AChE activity returned to
60-80% of control by 48 hr. MDA was slightly elevated in cortex at 1 and 2 hr post
dose. The phospholipid profile in brainstem at 2 hr showed a significant decrease in
phosphatidylcholine, a phospholipid unique to the mitochondrial membrane. Principal
component analysis (PCA) of 13C lipid spectra of brainstem at 2 hr showed a sep
aration between control and DFP-treated groups, indicating the potential to iden
tify distinct metabolite patterns between treatment groups. By understanding the
mechanisms of low level exposure to OPs and the impact on the metabolic func
tioning of specific brain regions, ongoing research can focus on the sensitive brain
areas to develop more effective therapeutic targets and preventative measures for
OP toxicity.

1257 ORGANOPHOSPHORUS-INDUCED DELAYED NEUROPATHY: AN EFFECTIVE THERAPEUTIC STRATEGY.
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UNESP São Paulo State University, Araraquara, São Paulo, Brazil. Sponsor: G.
DeOliveira.

Organophosphorus (OP), used as pesticides and hydraulic fluids, can produce acute
poisoning known as OP-induced delayed neuropathy (OPIDN), whose ef
fects take a long time to recover. This neuropathy is characterized by a distal ax
onopathy, Wallerian-like axonal degeneration. Owing to the large number of acci
dental or occupational cases of acute intoxication, some of which have been
misdiagnosed, a therapeutic strategy that prevents OPIDN developing would be in
valuable. Thus, the aim of this study was to investigate a strategy to reduce the clin
ical signs of OPIDN, based on the control of Ca2+ homeostasis. Forty-eight
isabrown leghorn hens, older 80 weeks and weighing about 2.4kg, were used. Levels
of Ca2+ in plasma, gastrocnemius muscle and sciatic nerve, sciatic nerve calcium
activated neutral proteinase (CANP) activity, lymphocyte neuropathy target es
terase (LNTE) and signs of neurotoxicity were monitored for 28 days following an
oral dose of tri-o-cresyl phosphate (TOCP), with and without treatment. The first
event after administration of TOCP (500 mg/kg, po), LNTE inhibition, was ob
served after 6h; the second event, a decrease in Ca2+ levels in the plasma and sciatic
nerve of hens occurred after 12h. The entrance of Ca2+ into the cell promoted an in
ecrease in CANP activity, 24h after introduction. Thus, the treatment consisted in
giving the hens nimodipine (1mg/kg, im) 12, 18 and 24h after TOCP and 30 min
utes later, calcium gluconate (5 mg/kg, iv), to restore Ca2+ homeostasis. There was
no significant (p<0.05) decrease of calcium in plasma, muscle and nerve after the
treatment, nor any significant increase in CANP activity in the sciatic nerve. Only
the positive control group had neurotoxicity behavioral scores different from zero.
In conclusion, these results show that this treatment improved both the biochemi
cal and clinical data and prevented the development of the neurodegenerative
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1258 ACUTE TOLUENE EXPOSURE ALTERS EXPRESSION OF GENES ASSOCIATED WITH SYNAPTIC STRUCTURE AND FUNCTION.
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Toluene (TOL), a volatile organic compound, is a ubiquitous air pollutant of inter
est to EPA regulatory programs. Whereas acute functional effects are well de
scribed, several potential modes of action in the CNS have been proposed.
Therefore, the genomic response to acute TOL exposure was investigated in rat
CNS to investigate further the potential pathways mediating its acute neurological
effects. Adult male Long-Evans rats inhaled clean air or 1000 ppm of TOL vapor
for 6 hrs. Brains were collected either zero or 18 hrs after removal from the exposure
chambers (n=6 / group / time). Total mRNA was extracted from the striatum of
each brain and hybridized to Rat 230A Affymetrix arrays. Statistical analyses using
ANOVA + FDR .05 showed 226 and 3525 genes altered in the TOL-exposed
groups relative to controls at the 0- and 18-hr times, respectively. Relative to con
trols, a common set of genes was differentially expressed at both time-points,
including genes for scaffold proteins that couple NMDA receptors to metabotropic
glutamate receptors and genes in pathways associated with long-term potentiation.
Analysis of the response at 0 hr showed differential effects of TOL on expression of
transcripts for calcium and potassium channel subunits, induction of genes for
early growth response (Egr2, involved in the onset of myelination) and GABAergic
neuronttransmission. The signature at 18 hrs showed marked effects on transcripts as
sociated with synaptogenesis, EphrinB receptors (associated with dendritic spine
morphogenesis), and GABA-receptor life cycling. These results provide evidence
that genomic responses begin during 6 hrs of TOL inhalation and are enhanced 18
hrs after removal. These results suggest that TOL alters transcription of proteins in
pathways associated with synaptic structure and function, and are consistent with previou
known effects of TOL on ion channels and synaptic transmission. (This abstract does not reflect EPA policy).

1259 TETRAMETHRIN AND DDT INHIBIT SPONTANEOUS FIRING IN CORTICAL NEURONAL NETWORKS.
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The insecticidal and neurotoxic effects of pyrethroids result from prolonged
sodium channel inactivation, which causes alterations in neuronal firing and com
munication. Previously, we determined the relative potencies of 11 type I and type
II pyrethroid insecticides using microelectrode array (MEA) recordings. In the pres
ent experiments, effects of the type I pyrethroid tetramethrin, or the organochlo
rine insecticide DDT, were examined on spontaneous spiking and bursting in pri
mary cortical cultures on MEAs. DDT is an organochlorine insecticide that acts on
voltage gated sodium channels in a manner similar to type I pyrethroids. We hy
pothesized that DDT’s effects on network activity would be similar to pyrethroids.
Effects of these compounds on spontaneous glutamatergic network spike and burst
rates were determined by recording activity in the presence of blockers of GABA re
ceptors. Spontaneous network spiking rate was inhibited by tetramethrin as well
DDT, with IC50 values of 3252 and 358 nM, respectively. However, at the concen
tration closest to the IC50, neither compound had significant effects on the number
of bursts per minute, percent of spikes in bursts, burst duration or interspike inter
val. Thus, like other pyrethroids, tetramethrin and DDT alter network firing rates,
but not the pattern of network bursting in glutamatergic networks. Tetramethrin
was more potent than the least potent pyrethroid (resmethrin, IC50 = 16860 nM)
examined to date, while DDT was similar in potency to the type II compound, β
-cyfluthrin (IC50 = 305 nM). Tetramethrin and DDT were 0.054 and 0.489, respec
ively, as potent as deltamethrin (relative potency = 1.0). These data demonstrate
that tetramethrin and DDT alter network firing rates in a manner similar to other
pyrethroids. In addition, these results expand our set of relative potency data for
pyrethroids to 13 chemicals (including DDT), providing one of the largest com
parisons of pyrethroid effects in mammalian neurons in vitro. (This is an abstract of
a proposed presentation and does not necessarily reflect Agency Policy).
1260 MANEB ENHANCES MPP+-INDUCED CYTOTOXICITY THROUGH ACTIVATION OF NF-KAPPA B IN PC12 CELLS.


Environmental factors have been associated with the pathogenesis of neurodegeneration. Exposure to maneb (MB), manganese ethylenebis-dithiocarbamate, has been linked to the development of parkinsonian-like symptoms in agricultural workers (Ferraz et al 1988; Meco et al 1994). Barlow et al. (2005) suggested MB has the ability to disrupt the antioxidant systems of dopaminergic cells. MB also can increase nitric oxide (NO) production by mediating inducible nitric oxide synthase (iNOS) activity. The production of NO can generate reactive oxygen species which in turn increase oxidative stress. NO can act as second messenger molecule to control important cellular processes by regulation of expression/activity of certain proteins such as NF-kappaB. NF-kappaB induction and the activation of NO synthase may contribute to disease progression. It is of interest to know whether MB may activate NF-kappaB, thereby enhancing MPP+ toxicity. In this study, PC12 cells were treated with PBS and MB (20 uM) for 1 h prior MPP+ (500 uM) treatment. After 16 h MPP+ treatment, cell viability was assayed by trypan blue exclusion. MB pretreated groups showed 47 % cell death after MPP+ treatment (MB treated groups as 100%) and the PBS pretreated group showed only 30 % cell death after MPP+ treatment (PBS treated groups as 100%). The result demonstrated that MB enhanced MPP+-induced cell death. Western blot analysis was performed to evaluate the NF-kappaB activation. Western blot data showed MB reduced the cytosolic NF-kappaB p65 level (15% reduction) after 3 h MB treatment. This implicated that MB activated the NF-kappaB and caused the nuclear translocation of NF-kappaB. This activation could be responsible for the MB synergistic effect on MPP+-induced cytotoxicity. However, further studies are needed to demonstrate whether NF-kappaB activation induced by MB is directly involved in the synergic effect of MB on MPP+-induced cell death.

1261 ANALYSIS OF C57BL/6 MICE AT 8 AND 16 MONTHS AFTER REPEATED DOSING OF PARAQUAT AND MANEB.


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Combined exposure to paraquat (PQ) and maneb (MB) in C57BL/6 mice has been hypothesized to be an animal model for Parkinson’s Disease. This study examines three doses and two time points to assess effects of repeated PQ+MB injections on a functional observational battery (FOB) with 12 open field arena observations, motor activity, and neurohistological endpoints. Male and female mice were dosed on PND 5-19 with 0, 3 or 10 mg/kg, 0.06/0.18 mg/kg, or 0.0067/0.0221 mg/kg PQ+MB respectively, and/or as adults twice weekly during weeks 27 to 31 of age. 8 mice at age with 10/30 mg/kg, 0.67/1.8 mg/kg, or 0.0067/0.0221 mg/kg PQ+MB. All animals were tested at 32 weeks, then set doses preweaning, as adult, or at both times were sacrificed at 33 weeks. A second set sacrificed at both times was held to 62 weeks and reassessed by the same FOB and motor activity tests, then sacrificed at 70 weeks. Brains of all animals were serially sectioned and every sixth section examined for cell degeneration and loss using GFAP, amonica silver (ACS) staining, and stereology of the substantia nigra pars compacta (SNpc). Gender effects were seen in the FOB, with high dose males showing increased incidence of tremor at 32 but not 62 weeks. High dose females showed increased horizontal activity and rearing at 62 weeks only. The high dose caused body weight loss in preweaning and adult males, and was lethal in males treated only as adults. Females did not show body weight-related effects at any dose, although brain weights were slightly reduced in high dose females. No treatment-related effects were seen across the whole brain using ACS and GFAP staining. Stereology is ongoing using StereoInvestigator v9.0 software to give a non-biased number of TH+ cells in the SNpc using the optical fractionator method.

1262 LOCOMOTOR ACTIVITY AS AN INDICATOR OF ACUTE CHLORPYRIFOS TOXICITY IN C. ELEGANS.

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Chlorpyrifos is a commonly used, broad-spectrum organophosphate insecticide that disrupts the nervous system. Symptoms of chlorpyrifos toxicity include muscle tremors, twitching, and in severe cases, paralysis and death. Neurotoxic effects of several organophosphates have been documented in the soil nematode Caenorhabditis elegans. We evaluated the locomotive activity of C. elegans after exposure to chlorpyrifos as a measure of neurotoxicity in these organisms. We hypothesized that there would be a dose-dependent decrease in locomotive activity in C. elegans exposed to chlorpyrifos. To test this hypothesis, we exposed worms to K-medium with or without chlorpyrifos (0.01 mM, 0.005 mM, or 0.001 mM) for 4 hours. We counted the number of body bends of worms as a measure of the rate of locomotion. The data indicate that nematodes exposed to 0.01 mM chlorpyrifos demonstrated fewer body bends than nematodes exposed to 0.001 mM after only 4 hours exposure, supporting our hypothesis. These results support the use of C. elegans as a model for early acute neurotoxicity testing.

1263 STRAIN AND DOSE-RELATED EFFECTS OF SUBCHRONIC CHLORPYRIFOS (CPF) EXPOSURE ON BIOMARKERS OF EXPOSURE AND OXIDATIVE STRESS IN RATS.


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Organophosphorus pesticide (OP)-induced neurotoxicity remains a significant public health concern, management of which is complicated by the lack of biomarkers that reliably identify at-risk individuals. We are developing a rat model of OP-induced neurotoxicity based on occupational exposures of Egyptian pesticide applicators to CPF to compare the predictive reliability of currently used biomarkers of OP exposure to versus biomarkers of oxidative stress. Long Evans (LE) is the rat strain used almost exclusively for behavioral studies; however, much of the data describing CPF metabolism in rats were obtained using Sprague Dawley (SD) rats. In this study, therefore, we compared CPF metabolism in these strains by measuring trichloro-2-pyridinol (TCPy), a CPF-specific metabolite, in daily urine and cholinesterase (ChE) in blood collected every 3 days and brain harvested at the end of a 7-day exposure to 0, 3 or 10 mg CPF/kg/d. Oxidative stress was assessed by measuring F2-isoprostanes in urine and brain. Our major findings are: 1) there are no effects of strain on dose-related responses; however, LE rats had increased urinary TCPy level and decreased brain ChE activity relative to SD rats; 2) peripheral measures of ChE activity and isoprostanes are predictive of CPF effects on these endpoints in the brain; 3) urinary TCPy does not predict blood ChE activity; 4) CPF exposure increases urinary and brain levels of F2-isoprostanes but correlations between F2-isoprostanes and either urinary TCPy or ChE activity were not evident. These data support the use of LE rats for biomarker studies and emphasize the need to evaluate the relationship between these biomarkers and neurobehavioral deficits in future studies. Supported by NIH grant #ES16308 (Anger and Lein, MPI)

1264 IN-COMMUNITY STUDY OF LONG TERM LOW-LEVEL EXPOSURE TO ORGANOPHOSPHATE PESTICIDES (OP) AND THE NEUROBEHAVIORAL EFFECTS AFTER THREE DECADES.


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Hula Valley in Israel has been extensively cultivated since 1957. Our studies in 1987-1991 assessed neurological effects of low-level long-term exposure to OP pesticides in 200 workers and residents in the valley. We are currently carrying on the work which started on the original cohorts to evaluate the extended outcome of these long-term OP exposure. The first study included a cohort of 60 individuals over 30 years of age. The second study which started on the original cohorts to evaluate the extended outcome of OP exposure to CPF in these individuals. The objective of this study was to examine the long-term effects of CPF exposure on neurological and cognitive function. We hypothesized that the OP exposed group would have a higher incidence of neurological and cognitive impairment compared to the unexposed group. The study included 51 subjects (27 CPF exposed and 24 non-exposed). The results of this study indicated no significant differences between the CPF exposed and non-exposed groups in terms of demographic characteristics, education level, and occupational exposure. The CPF exposed group had a significantly lower score on the Mini-Mental State Examination (MMSE) compared to the non-exposed group. The CPF exposed group also had a significantly lower score on the Digit Symbol Substitution Test (DSST) compared to the non-exposed group. Furthermore, the CPF exposed group had a significantly lower score on the Stroop Color-Word Test (Stroop) compared to the non-exposed group. These results suggest that long-term exposure to CPF may be associated with cognitive impairment. Further research is needed to confirm these findings and to explore the mechanisms underlying these effects.
1265  JP-8 JET FUEL EXPOSURE CAN SENSITIZE THE EAR TO SUBSEQUENT NOISE INDUCED HEARING LOSS.
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Previous research has suggested that subacute JP-8 jet fuel exposure (2000 mg/m³ 4 hr/day for 5 days) can enhance susceptibility to noise induced hearing loss produced by moderate (1 hr/day x 5 days 100 dB) noise. In this study we sought to determine whether or not combined JP-8 + noise exposure might make the inner ear more vulnerable to a subsequent loud noise exposure. This was accomplished by evaluating cochlear function as well as hair cell death in rats exposed to a single 4 hr. high intensity (105 dB) octave band noise exposure 4 days following the last exposure to JP-8 and mild noise. Auditory function was assessed within subjects using distortion product otoacoustic emissions (DPOAE) testing which evaluates outer hair cell function. Subsequently at four weeks after the last exposure, the cochleae were harvested from the rats for assessment of hair cell death. The data were compiled to compare to controls which received JP-8 and noise alone for 5 daily sessions. (Supported in part by VA RR&D grants 6006 and 4 hr noise treatment. However, in this study there was no clear increase in noise group that were treated only with the 5 daily low-moderate noise + the subsequent 4 hr noise treatment. However, in this study there was no clear increase in noise sensitivity in the rats treated for 5 daily sessions with the JP-8 + noise compared to noise alone for 5 daily sessions. (Supported in part by VA RR&D grants 6006 and 4613 and by the American Petroleum Institute)

1266  VACUOLAR CHANGES IN SENSORY NEURONS IN ORGANOPHOSPHATE-INDUCED DELAYED NEUROTOXICITY (OPIDN). INCIDENCE AND STRUCTURE.
Neuronal vacuolization is a lesion seen in several conditions, and has become an important consideration in neurotoxicological pathology. This study expands upon the work of Burgess et al. (2009) by assessing the nature and incidence of vacuoles associated with OPIDN in rat dorsal root ganglion (DRG) neurons. Young adult male Long-Evans rats were administered two organophosphates over a 63-day period, with sacrifice on days 28, 63 and 90. The test compounds were tri-ortho-tolyl phosphate (TOTP) given by gavage at 300 mg/kg on alternate days during the 14-28 and 49-63 day periods (14 total doses) and/or chlorpyrifos in two 60 mg/kg subcutaneous injections (on days 7 and 42). In addition 400 µg/ml of corticosterone in the drinking water was given for 90 days. There was TOTP-related OPIDN, including the presence of inhibition of brain neurotoxic esterase and bilateral myelinated fiber degeneration (Jortner et al. 2005). Lesions were most marked on day 90, thus this interval was selected to investigate the vacuolar change. By light microscopy, DRG neuronal cytoplasmic vacuoles were noted in 1/1244 cells for TOTP alone (n=4), 3/1021 cells for TOTP+chlorpyrifos (n=3), 10/1420 cells for TOTP+corticosterone (n=4), 5/1000 cells for TOTP+chlorpyrifos+corticosterone (n=3), 0/825 cells for controls (n=3). Electron microscopy demonstrated that these vacuoles were bound by a single, limiting membrane, surrounded by a region devoid of Nissl bodies. Protrusions of cytoplasm were seen budding into the vacuolar lumen; the latter of which appeared to contain fine, cellular debris. Vacuoles developed peripherally, and could often occupy most of the cellular volume. Regardless of degree of vacuolization, these lesions were not associated with any signs of cell death. These findings are consistent with those reported in cases of nerve crush injury, and are likely due to OPIDN-related axonal degeneration. Supported by USAMRMC DAMD17-99-1-9489.

1267  2, 5-HEXANEDIONE (HD) IMPAIRS THE CYTOSKELETAL PROTEIN INTERACTIONS OF MICROTUBULE ASSOCIATED PROTEINS (MAPS).
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We have hypothesized that HD causes nerve fiber atrophy by forming adducts with axonal proteins involved in cytoskeletal structure and function. To test this hypothesis, a computer-based quantitative morphometric method was used to measure cytoskeletal nearest-neighbor distances in spinal cord axons of moderately affected rats intoxicated at different dose-rates; i.e., 175 and 400 mg/kg/d. Consistent mean decreases (~34 %) in MT (microtubule)-MT distances were observed. Since this effect was shared by all MAPS, a targeted HD-induced axonal atrophy was used to characterize protein interactions in spinal cord homogenates of HD-intoxicated rats. Immunoblot analyses of proteins in the high-speed pellet fraction showed that HD did not alter MT yield or the co-sedimentation of motor proteins; i.e., KIF1A, KIF3, KIF5, Dynemin, and Dynamin I. However, HD-exposure caused selective decreases in the i.e., MAP1A, MAP1B-Heavy Chain, MAP2A/B, and Tau. No changes in protein associations were noted in corresponding supernatants. A corroborative study using a neurofilament (NF) “pull-down” assay found no changes in protein co-sedimentation in either supernatant or pellet. All NF subunits in both fractions from control rats, however, exhibited higher molecular weight (HMW) complexes. These HMW NF species were over-expressed in both high speed fractions from HD-intoxicated rats. To determine whether these axonal changes altered axonal transport, we analyzed the protein content of synaptosomes from striatum and cortex of HD-intoxicated rats. No changes in synaptobrevin, SNAP-25, GAPDH, COX-1, and NFs were identified suggesting that transport was not altered by HD; the HD-axonal atrophy was used to decrease in MT-MT distances, possibly through defects in the protein-protein interactions of MAPs. Supported by NIEHS grant R01 ESO7912-11.

1268  POSITIVE MODULATION OF THE NOVEL ANTI-APOTOTIC KINASE PKD1 CAN PROTECT DOPAMINERGIC NEURONS AGAINST OXIDATIVE DAMAGE IN PARKINSON’S DISEASE MODELS.
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Oxidative stress-induced apoptosis is known to play a causal role in the degenerative process in Parkinson’s disease (PD). We previously showed that PKCδ proteolytic activation is a key proapoptotic signaling mechanism that contributes to oxidative damage in PD models. The time course studies revealed that PKCδ activation precedes apoptotic cell death and that cells resisted early insults of oxidative damage, suggesting that some intrinsic compensatory response protects neurons from early oxidative insult. Therefore, the purpose of the present study was to characterize protective signaling pathways in dopaminergic neurons during early stages of oxidative stress. We uncovered that protein kinase D1 (PKD1) is activated via Fyn-PKCδ signaling pathway in a time dependent manner in cell culture and was not involved in PD. The PKD1 activation was maximal at the early stages of oxidative insult, while PKCδ proteolytic activation persisted over time and correlated with cytotoxicity. Importantly, PKD1 siRNA knockdown augmented oxidative stress-induced apoptosis, whereas overexpression of PKD1 full length and PKD1 constitutively active plasmids suppressed the apoptosis, suggesting an anti-apoptotic role for PKD1. A drug screening approach was conducted to identify a pharmacological activator of PKD1 that could help protect the dopaminergic neurons from oxidative damage independent of PKC δ cleavage. As proof of principle, we identified that rosiglitazone can activate PKD1 independent of PKCδ proteolytic activation and also protected against dopaminergic degeneration. Collectively, our results demonstrate that PKD1 activation acts as an intrinsic compensatory response against oxidative insult, while PKCδ proteolytic activation contributes to cell death. This may have important therapeutic implications for the treatment of PD.

1269  UNCOUPLING PROTEIN 2-INITIATED AUTOPHAGY PROMOTES CYANIDE-INDUCED NEUROTOXICITY: INVOLVEMENT OF BNIP3 UPRGREPATION.
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Cyanide is a potent neurotoxicant that inhibits cytochrome oxidase to initiate mitochondrial-mediated cell death. Our previous studies showed that cyanide produced apoptosis or necrosis in select brain areas. The mode of death was dependent on cell type and level of insult. Uncoupling protein 2 (UCP-2) upregulation enhances cyanide-induced neurotoxicity by increasing ROS generation and mitochondrial dysfunction. This study shows that UCP-2-initiated autophagy contributes to the cyanide response. A tet-on UCP-2 inducible N27 cell line was used to control UCP-2 expression during cyanide toxicity. Compared to wild type cells, tet-on UCP-2 cells were more sensitive to cyanide and died by autophagy. Following cyanide exposure, the tet-on UCP-2 cells upregulated beclin-1, a critical mediator of autophagy. Transfection with GFP-LC3 (autophagy marker) showed that increased UCP-2 expression stimulated autophagosome formation, as reflected by punctate GFP-LC3 green fluorescence. The role of autophagy was further involved in the mitochondrion-mediated cell death.
Parkinson's disease is characterized by a gradual degeneration of dopaminergic neurons in the substantia nigra. Dopaminergic neurons are continuously exposed to elevated oxidative stress conditions due to the unstable neurotransmitter dopamine that can easily undergo oxidation to form superoxide and a quinone-form capable to react with cysteine residues in proteins or with glutathione to form dopamine-conjugates. For investigations on the molecular events occurring under these conditions, as well as for the validation of potential pharmacological interventions, an experimental human in vitro model that closely resembles the characteristics of dopaminergic neurons in vivo was established.

LUHMES cells are conditionally immortalized human fetal mesencephalic cells that acquire a dopaminergic phenotype following differentiation for 6 days. LUHMES were characterized with respect to their response toward the parkinsonian toxin MPP⁺ that not only inhibits mitochondrial complex I but can also trigger the release of vesicular dopamine. In LUHMES, MPP⁺ caused a time-dependent degeneration of neurites, accompanied by a loss of cellular ATP and GSH, and increased formation of radical species. The neurodegenerative effects observed were partially prevented by co-incubation with the mixed lineage kinase inhibitor CEP1347, by inhibition of poly-ADP-ribose polymerase (PARP), or by desferrioxamine and ascorbic acid. Inhibition of the proteasomal system or the siRNA-mediated knockdown of alpha-synuclein also demonstrated protective effects, suggesting that the LUHMES/MPP⁺ model reflects the major in vivo features of parkinsonian brains such as energy impairment, oxidative stress, and proteolytic stress.

Parkinson’s Disease (PD) is a progressive neurodegenerative disorder, characterized by the loss of dopaminergic neurons. Dopamine (DA), an important neurotransmitter, undergoes catabolism to form 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is structurally analogous to DA, but is a reactive intermediate; therefore, it has the potential to interact with proteins containing DA-binding sites. Recent studies have shown that DOPAL, at pathological levels, modifies proteins in dopaminergic neurons. Currently, the identity of these target proteins and the effect on function are unknown. Therefore, it is hypothesized that DOPAL modifies and inhibits enzymes that are important to dopamine biosynthesis and trafficking. Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in DA synthesis, converting tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA). Nerve growth factor inhibits enzymes that are important to dopamine biosynthesis and trafficking. The involvement of BNIP3, a BH3-only Bcl-2 protein, in the UCP-2 enhancement of cyanide neurotoxicity involves the autophagic mode of cell death and BNIP3 is a mitochondrial mediator of the neurotoxicity (Supported by NIH grant ES04140).
INTERACTION OF AN ENDOGENOUS NEUROTOXIN, 3,4-DIHYDROXYPHENYLACETALDEHYDE, AND GLIAL CELLS: METABOLISM, ACTIVATION, AND TOXICITY.

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The cause of the hallmark dopaminergic cell death of Parkinson’s Disease (PD) is unknown, but recent research indicates oxidative stress and the endogenous neurotoxin, 3,4-dihydroxyphenylacetaldehyde (DOPAL), to play roles in the disease pathogenesis. DOPAL is generated from dopamine (DA) by monoamine oxidase and oxidized to 3,4-dihydroxyphenylacetic acid by aldehyde dehydrogenase. The dopamine metabolite is highly toxic to dopaminergic cells and needs to be rapidly metabolized to prevent toxicity. Non-neuronal cells express high levels of MAO-B, but formation and metabolism of DOPAL within these cells has not previously been measured. Microglial cells have been shown to be activated in neuronal regions containing high MAO-B activity via DA-protein adducts, specifically in the striatal region containing the substantia nigra; however, the mechanism of this activation has not been demonstrated, and could be due to DOPAL or DOPAL-protein adducts. Activated microglia cause injury to dopaminergic neurons via a host of mechanisms, including reactive oxygen species production, release of cytokines, and phagocytic activity. The ability of DA, DOPAL, and other DA metabolites to activate BV-2 microglial cells was previously unknown, but DOPAL-mediated activation has been demonstrated in this work, as measured by TNF-α secretion. Metabolism of DA and DOPAL and toxicity of DOPAL, as analyzed by the MTT and LDH assays, were determined for glial cells. It was found that glial cells metabolize DA to DOPAL, and DOPAL further to DOPAC, and in addition, greater cytotoxicity was observed for those cells treated with DOPAL compared to DA. DOPAL-mediated activation of microglia demonstrated in this study could represent a mechanism for inflammation and dopaminergic cell death detected in patients with PD.

OXIDATION OF 3,4-DIHYDROXYPHENYLACETALDEHYDE AND REACTIVITY WITH PROTEIN NUCLEOPHILES.

D. G. Anderson, V. Florang and J. A. Doorn. Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA.

Parkinson’s disease (PD) is a progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra of the brain. Among the many factors believed to be involved in the pathogenesis of PD are aberrant dopamine (DA) metabolism and trafficking and the inherent toxicity of DA and certain DA metabolites. One mechanism of toxicity for DA centers on its ability to autotoxicize to an electrophile. This oxidizes DA, leading to oxidation, redox cycling, and reactive oxygen species production, all known mechanisms of toxicity. 3,4-Dihydroxyphenylacetaldehyde (DOPAL) is the oxidative metabolite of DA and is significantly more toxic than its parent amine. Mechanisms of toxicity for DOPAL include hydroxyl radical formation, and our group has shown that DOPAL is capable of forming protein adducts via a hypothesized Schiff-base mechanism. Little is known about the ability of DOPAL to undergo oxidation to a quinone similar to DA. However, the formation of such a species could be key for understanding DOPAL toxicity and could potentially be involved in DOPAL-induced protein reactivity. Experimental evidence indicates that DOPAL is capable of such an oxidation, and that oxidized DOPAL reacts readily with cellular nucleophiles such as thiols. Various factors leading to DOPAL oxidation have been investigated (chemical, transition metal, enzymatic) in order to better understand the potential role of this highly electrophilic species in relevant biological systems. Also, the role of DOPAL as a substrate for prostaglandin H synthase-2 is demonstrated for the first time. Furthermore, kinetic studies indicate that oxidation may be involved in the reactivity of DOPAL with protein-type nucleophiles such as amino acids, and could therefore be involved in laboratory observed protein cross-linking. Investigating such species is important for determining their biological relevance and involvement in PD.

RESVERATROL PROTECTS AGAINST MPP+ AND METHAMPHETAMINE NEUROTOXICITY BY MODULATING THE PKC-DELTA APOPTOTIC SIGNALING PATHWAY AND MICROGLIAL ACTIVATION.

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Resveratrol (3, 4', 5'-trihydroxy stilbene) is a phytoalexin that has been shown to produce beneficial effects in various disease models. Generally recognized as a potent antioxidant, resveratrol has also been shown to modulate various signaling molecules including SIRT-1. We hypothesized that resveratrol could have a dual neuroprotective function in models of dopaminergic neurotoxicity by protecting against neuronal apoptosis and by suppressing microglia mediated neurotoxicity. Specifically, we examined whether i) resveratrol protects against dopaminergic neurodegeneration induced by the Parkinsonian toxics MPP+ and methamphetamine (Meth), ii) resveratrol attenuates microglial activation, and iii) resveratrol modulates the PKCδ dependent proapoptotic signaling in dopaminergic neuronal cells. Resveratrol treatment in the N27 mesencephalic dopaminergic neuronal cell model significantly attenuated MPP+ and Meth-induced cell death in a dose-dependent manner. This protective effect was accompanied by attenuation of neurotoxicant-induced caspase-3 enzyme activity as well as proteolytic cleavage of proapoptotic kinase PKCδ. Interestingly, resveratrol effectively blocked PKCδ expression and MPP+ induced PKCδ proteolytic activation. Furthermore, we confirmed the neuroprotective effect of resveratrol in mouse primary mesencephalic cultures by determining the number of TH positive neurons. Resveratrol also attenuated LPS-induced nitric oxide production and proinflammatory cytokines in BV-2 microglial cells in a dose-dependent manner, indicating that resveratrol can also suppress microglia activation and the subsequent neuroinflammatory response. Collectively, our results demonstrate that resveratrol protects against the degenerative process in dopaminergic neurons by modulating the PKC-δ dependent apoptotic pathway as well as by suppressing microglia mediated neurotoxicity (NS 58644 and NS65167).

EFFECTS OF MEMANTINE ON NEURONAL OXIDATIVE DAMAGE.

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Increased innate immune response and excitatory stimulation contribute to neurodegeneration inherent to Alzheimer’s disease, HIV-associated dementia, ischemic stroke, head trauma, Huntington’s disease as well as some forms of epilepsy and cerebral palsy. Findings from patients and animal models have widely supported the hypothesis that neuronal oxidative damage is a major effector contributing to neurodegeneration. Therefore, we investigated whether memantine, a N-methyl D-aspartate (NMDA) receptor antagonist, can effectively suppress oxidative damage and neurodegeneration in activated innate immunity (lipopolysaccharide, LPS) and excitotoxic (kainic acid, KA) injury. Mice exposed to intracerebroventricular (icv) administration of lipopolysaccharide (LPS; 5 μg/5 μl for 24 hours) or KA (1 nmol/5 μl for 30 minutes) showed significant increase in biomarkers of oxidative damage, F2-isoprostanes (F2-IsoPs, 155% and 199%, respectively). At the same times, hippocampal pyramidal neurons showed significant reductions in dendritic length (> 66%) in both disease models compared to control (100%). Pretreatment with memantine (5 mg/kg, ip) was more effective in suppressing oxidative damage associated with activation of cerebral innate immunity than excitotoxicity caused by KA. Importantly, peripherally administered memantine (5 mg/kg, ip) completely blocked the icv LPS- and KA-induced decrease in the dendritic length of hippocampal pyramidal neurons. A lower dose of peripherally administered memantine (1 mg/kg, ip) was equally effective in suppressing oxidative damage and dendritic degeneration in CA1 hippocampal pyramidal neurons associated with activated innate immunity model, but failed to reverse the KA-induced decrease in dendritic length. These data confirm that memantine (5 mg/kg) effectively protects hippocampal neurons from oxidative damage in 2 distinct disease models, meriting future studies on conditions in which it can optimally mitigate neurodegenerative processes (Supported by Forest Laboratories, NAM 38 to DM).
1278 PINK1 AND MITOCHONDRIAL DYNAMICS: A NEW PERSPECTIVE OF MITOCHONDRIAL DYSFUNCTION IN PARKINSON'S DISEASE?

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Mutations in the mitochondrial encoded protein PTE-N-inducible putative kinase 1 (PINK1) cause autosomal recessive Parkinson's disease (PD). In mammalian cells, mutant PINK1 has been reported to promote fission or inhibit fusion on mitochondria; however, the mechanism by which this process occurs remains elusive. Using an inducible expression system in mammalian dopaminergic neurons, we report here that human mutant PINK1 (L547P and W437X) mediates an overall fission effect by increasing the ratio of mitochondrial fission over fusion proteins, leading to excessive dysfunctional fragmented mitochondria. Knocking down endogenous PINK1 produces similar effects. In contrast, over-expressing human wild type PINK1 produces a pro-fusion effect by increasing the ratio of mitochondrial fusion/fission proteins, without resulting in functionally compromized mitochondria. Parkin knockdown blocks the imbalance in fission/fusion proteins—suggesting PINK1 and parkin maintain proper mitochondrial function and integrity via this mitochondrial machinery. Through genetic manipulations and pharmacological treatments, we demonstrated that mitochondrial fission, a key feature of mitochondrial dysfunction, may be responsible for the loss of mitochondrial membrane potential, which is a crucial determinant of mitochondrial fission. These findings are in agreement with the hypothesis that loss of mitochondrial membrane potential may lead to the loss of dopamine neurons, thus providing a potential therapeutic target for the treatment of Parkinson's disease.

1279 FUNCTIONAL ROLE OF THE NEUROTOXICITY BIOMARKER PROTEIN TSPO/PBR IN PRIMARY MICROGLIA.

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Translocator protein-18 kDa (TSPO), previously called the peripheral benzodiazepine receptor (PBR), is a glia protein that has been extensively used as a biomarker of brain injury in a variety of animal models of neurotoxicity and in humans. Inflammation is a hallmark of microglial activation. While TSPO is a validated biomarker of neurotoxicity, the functional role of TSPO in glial cells is not known. In this study, we examine the effect of TSPO activation by the TSPO-specific ligands R-PK11195 (PK) and Ro5-4864 (Ro) in primary microglia. Exposure to different concentrations (1-1000 nM) of PK for 24 hrs resulted in increased expression of intracellular reactive oxygen species and decreased mitochondrial fission. TSPO activation regulates mitochondrial processes that are essential to microglial function. Further, when microglia are activated by an inflammmogen, TSPO activation enhances IL-1β release and increases microglial apoptosis. These findings suggest that TSPO plays a critical role in cellular functions that are essential to mount a microglial response to the disruption of brain homeostasis. They also indicate that under pathological conditions, TSPO activation may be responsible for the elimination of activated microglia via apoptosis. [Supported by NIEHS-ES07062 to TRG and NIEHS-T32 ES07141]

1280 OVAIRECTOMY POTENTIATES REDUCTIONS IN STRIATAL NERVE TERMINAL DOPAMINE LEVELS AFTER CHRONIC PCB EXPOSURE.

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Estrogen is suggested to protect against Parkinson's disease (PD) because reductions in estrogen levels by ovariectomy increases the risk of PD in women, and hormone replacement therapy protects against PD. Estrogen also protects against loss of dopaminergic function in animal models of PD. Polychlorinated biphenyls (PCBs) are dopaminergic toxicants implicated in PD, and women occupationally exposed to PCBs have higher PD mortality than males (Epidemiology, 2006, 17:8-13). We have investigated whether reductions in female ovarian hormones, induced by ovariectomy (OVX), modulate the effects of chronic exposure to PCBs (A1254) on dopamine function in C57BL/6J mice. Female mice underwent OVX or sham surgery at 11 weeks of age. After 2 weeks' recovery mice were exposed to 500 ppm A1254 or corn oil (vehicle) for 70 days. After sacrifice, DAT activity was assessed by [3H] DA uptake and levels of DA and DA metabolites were analyzed by HPLC in striatal synaptosomes.

A1254 significantly reduced synaptosomal DA levels relative to vehicle exposed animals in OVX, but not in sham operated females, suggesting that ovariectomy sensitizes female mice to A1254. DA turnover, assessed as DOPAC/DA ratios, was increased by ovariectomy, indicating that loss of gonadal hormones increased DA metabolism. In contrast, DA turnover was not altered by A1254 exposure, A1254 exposure competitively inhibited DA uptake via DAT, indicated by increased DAT Kd values, relative to controls in both sham and OVX animals. These observations suggest that the 'double hit' of OVX and A1254 exposure, increasing DA turnover and inhibiting DA reuptake into the nerve terminal, respectively, lead to reduced DA levels in striatal nerve terminals. These findings are in agreement with the hypothesis that loss of ovarian hormones increases sensitivity to environmental contaminants, and suggests that postmenopausal women may be more sensitive to PCBs than premenopausal women. Supported by NIH grant RO1ES014675 to RFS.

1281 TANESPIMICYCIN BLOCKS BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY IN RATS.


Tanespimycin (TAN) is a Heat-Shock Protein 90 inhibitor currently in Phase 3 clinical trials with bortezomib (BTZ) for the treatment of multiple myeloma (MM). While BTZ-induced severe peripheral neuropathy is observed in 8-12% of patients with MM, no severe peripheral neuropathy was observed in a Phase 1/2 trial in MM patients treated with BTZ and TAN. The potential neuroprotective effect of TAN was confirmed in an initial TAN/BTZ combination study in rats utilizing a sensory threshold endpoint (ie, mechanical allodynia) wherein BTZ-induced hypernociception, consistent with a peripheral neuropathy, was ameliorated by co-administration of TAN. We extend these findings in the same model by exploring exploring electrophysiologic and histopathologic endpoints. Male Sprague-Dawley rats were intravenously administered BTZ and/or TAN at clinically relevant doses (ie, 0.22 mg/kg and 20 mg/kg, respectively) twice weekly for 5 to 8 weeks; both preventative and reversal paradigms were explored. After 5 weeks of dosing with BTZ alone, nerve conduction velocity (NCV) in the caudal nerve was significantly reduced compared to age-matched control values (p < 0.05). However, when BTZ and TAN were co-administered, the deficit in caudal nerve NCV was prevented and velocities were indistinguishable from findings in age-matched controls. Histopathologic evaluations, including electronic microscopy and immunohistochemistry, are ongoing. These findings confirm that TAN prevents BTZ-induced neuropathy in rats, consistent with the observation of less severe neuropathy in humans. To our knowledge, this is the first drug demonstrated to protect against BTZ-induced neuropathy in animal models, with clinical evidence to support a bench-to-bedside correlation.

1282 THE PROGRESSION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALITIS IS DEPENDENT ON NOD2 AND RICK ACTIVATION IN MICROGLIA REPRESENTING A NOVEL THERAPEUTIC TARGET FOR MULTIPLE SCLEROSIS.

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The innate immune system is required for host defense to recognize invading pathogens and to eliminate them. Recent work has identified a group of cytosolic pattern recognition receptors called the NOD-like receptor (NLR) family of proteins. NOD1 and NOD2 are NLR proteins which are activated by fragments of peptidoglycan. Following direct or indirect activation of NOD2 by muramyl dipeptide (MDP), a CARD domain is exposed, recruiting the kinase RICK via homotypic CARD interactions. RICK activation results in MAPK pathway and NF-kB activation. Multiple sclerosis (MS) is an autoimmune disease in which T cells attack oligodendrocytes and strip neurons of the myelin sheath required to propagate electrical signals. Peptidoglycan from a bacterial infection or gut microflora is a common trigger for MS. It is hypothesized that MS arises from an infection or gut dysbiosis that triggers the release of peptidoglycan either directly or indirectly by stimulating RICK activation. In this study, we modeled experimentally induced autoimmune encephalitis by immunizing mice intraperitoneally with MOG (100–500 ng/mouse) and oral administration of peptidoglycan (MDP, 500 ng/mouse). The mice were observed for 28 days and histopathology was performed. The mice were imaged with T2, T1, and FLAIR MRI sequences. The brain samples were analyzed for RICK and NOD2 expression by immunohistochemistry. The mice that were treated with MDP had higher expression of RICK and NOD2 in the brain compared to the control group. These results suggest that RICK and NOD2 activation play a role in the progression of experimental autoimmune encephalitis and represent a novel therapeutic target for multiple sclerosis.
carried to the brain in primates during experimental autoimmune encephalitis (EAE), the animal model of MS. However, the role of PGN or its cytosolic sensor (NOD2) in the progression of EAE is unknown. Both NOD2-/- and RICK-/- mice were protected from the progression of EAE. The peripheral activation of myelin oligodendrocyte glycoprotein (MOG)-specific T cells was similar in WT, NOD2-/- and RICK-/- mice. However, the number of CD4 T cells and MOG-specific T cells in the central nervous system (CNS) at the peak of disease severity was slightly reduced in NOD2-/- mice and significantly lower in RICK-/- mice. Additionally, the activation of antigen-presenting cells in the CNS was slightly reduced in NOD2-/- mice and significantly reduced in RICK-/- mice. The results in NOD2-/- mice are moderate likely due to microglia being activated by NOD1. The reduced activation of microglia in RICK-/- mice results in less re-priming of T cells and MOG-specific T cells. NOD2-/- and RICK-/- mice. However, the number of CD4 T cells and MOG-specific T cells in the CNS was slightly reduced in NOD2-/- mice and significantly lower in RICK-/- mice. Additionally, the activation of antigen-presenting cells in the CNS was slightly reduced in NOD2-/- mice and significantly reduced in RICK-/- mice. The results in NOD2-/- mice are moderate likely due to microglia being activated by NOD1. The reduced activation of microglia in RICK-/- mice results in less re-priming of T cells once they infiltrate the CNS, resulting in their death. Thus, RICK inhibition in microglia represents a novel therapeutic strategy for the treatment of MS.

6-OHDA, and Mn2+ similarly inhibit [Ca2+]i waves, however Mn2+ requires a higher concentration to produce equivalent calcium wave inhibition. These findings indicate that endogenous and exogenous chemicals that are structurally diverse but that have actions structurally diverse but that have actions similar to those of BACE, inhibit physiological calcium signaling in astrocytes. Because these astrocytic signals are critical to regulation of rCBF, these data suggest a new target for neurotoxins that may provide insight into mechanisms of decreased cerebral blood flow that may contribute to the onset of neurological disease.

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Neuroinflammation is associated with loss of dopaminergic neurons in Parkinson’s disease (PD) but there are no approved therapeutics that block this phenotype. Activation of microglia and astrocytes leads to overproduction of inflammatory mediators such as tumor necrosis factor and nitric oxide that damage dopaminergic neurons. Thus, increased neuronal protein nitration is detected post-mortem in PD patients. Expression of nitric oxide synthase (NOS2) and other inflammatory genes in astrocytes is mediated largely by the transcription factor, NF-κB, thought to be a key pathway regulating inflammatory signaling in glial cells. We therefore postulated that inhibitors of NF-κB signaling may have potential therapeutic utility in preventing inflammatory expression of NOS in astrocytes. To address this hypothesis, we examined the anti-inflammatory capacity of a representative diindolylmethane-derived compound (cDIM) in primary astrocytes, as well as its neuroprotective efficacy in vitro. Low doses of cDIM globally suppressed MPTP-induced expression of NF-κB-regulated genes in a qPCR array study and also inhibited dose-dependent inhibition of Nos2 mRNA expression. cDIM treatment prevented MPTP-induced protein nitration in astrocytes that mirrored the effect of the NOS2 inhibitor, AMT, indicating the functional effect of inhibiting NOS2 activity. Using live-cell fluorescence imaging in transgenic astrocytes expressing an NF-κB reporter construct, it was discovered that low concentrations of cDIM also directly prevented NF-κB-dependent gene expression. In co-culture studies with primary astrocytes and striatal neurons, cDIM pretreatment prevented astrocyte-dependent neuronal apoptosis, indicated by decreased activation of caspases and annexin V staining. Collectively, these data suggest that use of pharmacologic inhibitors of NF-κB could be effective in mitigating the effects of neuroinflammation by activated glial cells. Work supported by: Michael J. Fox FDN for Parkinson’s Disease Research (RBT).

B. Trout, K. M. Streifel and R. B. Tjellken, Environmental Radiological and Health Science, Colorado State University, Fort Collins, CO.

Calcium signaling throughout networks of astrocytes is initiated by synaptic activity and in order to increase regional cerebral blood flow (rCBF) in response to the local demand for oxygen and glucose. This increase in intracellular calcium [Ca2+]i in perivascular astrocytes causes a release of vasoactive factors that cause a rapid, local dilation of arterioles. Depreciations in rCBF are well described in patients with various neurodegenerative diseases but the mechanisms underlying these decreases are unknown. To examine the possible contribution of astrocytic dysfunction to this phenomenon, we postulated that several structurally diverse cationic neurotoxins of the basal midbrain would inhibit transmitter-induced calcium signaling in cultured astrocytes: MPP+ , the active metabolite of the model parkin-sonian neurotoxicant, 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP); Paraquat (PQ); 6-Hydroxydopamine (6-OHDA); and Manganese (Mn2+). Using calcium imaging in primary cultured cortical astrocytes, we investigated the effect of acute treatment with each neurotoxicant on ATP-induced intracellular calcium transients. We observed a dose dependent increase in ATP-induced [Ca2+]i transients with acute application of PQ, 6-OHDA and MPP+. In addition, mechanically-induced intercellular [Ca2+]i waves were inhibited in the presence of MPP+, an effect that was reversible following washout of the compound. Like MPP+, PQ,

1283 BETA-SECRETASE GENE EXPRESSION AND ACTIVITY IN MURINE GT1-7 HYPOTHALAMIC NEURONS EXPOSED TO CHOLESTEROL SECOALDEHYDE.

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Previous studies from our laboratory have demonstrated that 3β-hydroxy-5-oxo-5,6-secocholestan-6-al (cholesterol secoaldehyde or ChSeco), an oxysterol known to be formed at inflammatory sites as a result of myeloperoxidase/H2O2/CCL3- or singlet oxygen-mediated oxidation of cholesterol, promotes Aβ aggregation in GT1-7 hypothalamic neurons. Since Aβ aggregation critically depends on the cleavage of amyloid precursor protein (APP), and since increased activity and expression of secretases are often seen in Alzheimer’s disease, we examined the activity of β-secretase in neuronal cells exposed to low levels of ChSeco (1-5 μM) for 24 h. The results showed that the β-secretase activity was not altered in response to the exposure of ChSeco. Also, co-incubation of the ChSeco (5 μM)-exposed neuronal cells with Trolox (0.2 mM) caused a little or no change in the activity of β-secretase. To examine the significance of these results at gene expression level, we isolated total RNA from the control and the ChSeco-treated neuronal cells, prepared cDNA, and performed RT-PCR analysis for β-secretase isozyme-1, APP, and β-actin (housekeeping gene). It was found that the mRNA levels of β-secretase isozyme-1 and APP in the ChSeco (0-2.5 μM)-exposed neuronal cells were about the same as those in the control cells that were never exposed to ChSeco. While the results need further confirmation employing higher but sub-cytotoxic concentrations of ChSeco and the measurement of other secretases and, possibly, the proteosomal results need further confirmation employing higher but sub-cytotoxic concentrations of ChSeco and the measurement of other secretases and, possibly, the proteosomal activation of antigen-presenting cells in the CNS was slightly reduced in NOD2-/- mice and significantly reduced in RICK-/- mice. The results in NOD2-/- mice are moderate likely due to microglia being activated by NOD1. The reduced activation of microglia in RICK-/- mice results in less re-priming of T cells once they infiltrate the CNS, resulting in their death. Thus, RICK inhibition in microglia represents a novel therapeutic strategy for the treatment of MS.

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1284 STRUCTURALLY DIVERSE CATIONIC NEUROTOXICANTS ATTENUATE ATP-DEPENDENT CALCium SIGNALING IN ASTROCYTES.

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High incidence of Parkinson’s disease (PD) is correlated with a pesticide usage in agricultural areas. Mitochondrial inhibition, which also can cause oxidative stress, may lead to dopaminergic (DAergic) neuronal death and subsequent neuro muscular dysfunction associated with PD. In order to test the hypothesis that mancozeb (MZ: manganese/zinc ethylene-bis-dithiocarbamate) may contribute to the high incidence of PD, we chronically exposed various strains of the nematode C. elegans to multiple concentrations of MZ. Image analysis of fluorescent photomicrographs of NW1229 (all neurons tagged with GFP) were compared to control (CN: 407/12a1578) worms. There was no difference among treated groups (p=0.05; 0.1, 1.0, 1.5, 1.6 or 1.7%). Furthermore, pixel number was also decreased (p=0.01) in the dorsal nerve cord in all treatment groups (≈6202±1071) compared to CN (13236±1444). In order to investigate the role of oxidative stress, we used N2 (wild type) and RB1197 (catalase knock-out) strains. Both were treated with varying concentrations of MZ, then placed on nematode growth media (NGM) plates for 24 h. Following removal of MZ, N2 worms were incubated in dihydroethidium (DHE), a fluorescent dye that binds DNA in the presence of superoxide. When quantification of fluorescence was adjusted for lethality, there was a statistically significant increase (p=0.001) at 1.5% MZ (299±11) compared to CN (116±1). RB1197 worms were counted 24 h post-
treatment for number of live worms. Surprisingly, RB1197 worms showed less lethality at 1% MZ (69.0±3.8% live worms) compared to N2 (47.2±1.2%), suggesting hydrogen peroxide is not the major reactive oxygen species (ROS). Taken together, these data demonstrate that observed neurodegeneration in *C. elegans* is not dose dependent and likely results from increases in ROS, particularly superoxide.

### 1287 NEURONAL DEGENERATION FOLLOWING TOUCHDOWN EXPOSURE IN *C. ELEGANS* MAY BE DUE TO OXIDATIVE STRESS.

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Incidence of Parkinson's disease (PD), resulting in dopaminergic neurodegeneration, has been positively correlated with pesticide usage. Data suggest damage results from increased reactive oxygen species (ROS) and mitochondrial dysfunction. In these studies, three *C. elegans* strains, N2 (wild type), NW1229 (pan-neuronal GFP) and RB1197 (catalase KO), were exposed to the glyphosate-containing pesticide Touchdown (TD) to address the hypothesis that neuronal damage following TD exposure may result from ROS formation. Synchronous L2 worms were acutely treated with varying concentrations of TD and assessed for multiple endpoints. NW1229 treated with 10% TD (LC50 =7.3%; 95% CI=5.56-9.52; t2 =0.6886), resulted in photomicrographs suggesting neurodegeneration compared to control (water; CNs). Subsequent experiments in N2s with tetramethylrhodamine ethyl ester (TMRE), a fluorescent dye taken up by mitochondria with intact membrane potentials (Δψ), demonstrated mitochondrial inhibition (ANOVA p<0.0043) in TD versus CN worms, with the greatest inhibition at 15% TD (p<0.01). To determine which ROS might contribute to neurodegeneration, RB1197 worms were treated with varying concentrations of TD. Interestingly, RB1197 appeared to be less sensitive to TD treatment (61.2±16.8% live worms) compared to N2 (43.5±12.4% live worms) at 10% TD. N2 worms also were incubated with dihydrothreitol (DHE), a fluorescent dye that binds DNA in the presence of superoxide. Quantification indicated a positively correlated trend (p<0.20) of increased luminosity in treated versus CN worms. Furthermore, RB1197 worms showed a statistically significant increase in luminosity (p<0.001) for 10% TD versus CN. Taken together, these data suggest *C. elegans* exposed to the glyphosate-containing pesticide TD demonstrate neurodegeneration that may result from mitochondrial inhibition and/or increased ROS production. Since number of live worms does not differ significantly between N2 and RB1197, it is likely that hydrogen peroxide is not the primary oxidant. Rather, DHE data suggest superoxide may be the primary oxidative species.

### 1288 PYRETHROID PESTICIDE-INDUCED APOTOPSIS: ROLE OF THE ER STRESS PATHWAY.

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Exposure to the pyrethroid pesticide deltamethrin has been demonstrated to cause apoptosis both in vitro and in vivo (Wu and Liu, 2000; Elwan et al., 2006). However, the molecular pathways leading to deltamethrin-induced apoptosis have not been established. Here, we sought to identify the molecular pathways by which pyrethroid pesticide Touchdown (TD) induce number of live worms. Surprisingly, RB1197 worms showed less lethality at 1% MZ (69.0±3.8% live worms) compared to N2 (47.2±1.2%), suggesting hydrogen peroxide is not the major reactive oxygen species (ROS). Taken together, these data demonstrate that observed neurodegeneration in *C. elegans* is not dose dependent and likely results from increases in ROS, particularly superoxide.

### 1289 ACRYLAMIDE ADDUCTION AND S-NITROSYLATION OF NEURONAL PROTEINS.

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Acrylamide (ACR) is a neurotoxin that forms irreversible adducts on specific cysteine (Cys) residues of proteins. Endogenous nitric oxide (NO) has also been shown to reversibly modify susceptible Cys-containing proteins to modulate function. Because both ACR and NO are likely to modify reactive thiols, i.e. those with a low pKa, S-nitrosylation (SNO) Site Identification or SNOSID was used to identify putative SNO-Cys sites on NO-regulated target proteins in rat primary immortalized mesencephalic dopaminergic cells (N27). These sites were then compared to those modified by ACR. Using SNOSID, we have identified proteins with susceptible Cys containing peptides such as annexin A1 (3 peptides), 60s ribosomal protein L4 (5 peptides), proliferating cell nuclear antigen (3 peptides), and DEAD (Asp-Glu-Ala-Asp) box polypeptide (3 peptides), suggesting that these proteins are targets for S-nitrosylation. Following exposure to ACR, ACR adducts were also identified on A modified peptide from annexin A1, suggesting that NO and ACR can target identical Cys-groups. To link ACR-mediated Cys adduction to protein function, the effect of ACR on glyceroldehyde 3-phosphate dehydrogenase activity and adduct formation was investigated. GAPDH enzyme activity was significantly decreased in a dose-dependent manner by exposure to ACR. Other type 2 alkenes (acrolein and methyl-acrylate) were also evaluated for the ability to inhibit GAPDH and did so in a dose dependent manner. The ability of type II alkenes to inhibit GAPDH activity was similar to ACR. Methyl Acrylate < Acrolein which is similar to their reactivity with cellular thiols. These data suggest that ACR and NO target similar proteins leading to modulation of protein activity that is mechanistically important for ACR neurotoxicity. These studies were funded by ROI ES03830-20.

### 1290 ROLE OF THE DIVALENT METAL TRANSPORTER, GLUTATHIONE-S-TRANSFERASE PI, AND ER STRESS RESPONSE PROTEINS IN *C. ELEGANS* MODELS OF PARKINSON'S DISEASE AND MANGANISM.

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Manganese (Mn) neurotoxicity resembles a number of aspects of the dopamine (DA) neuron degenerating disorder Parkinson's disease (PD). Both PD and manganese is characterized by motor deficits and damage to substantia nigra and other basal ganglia nuclei, and dopamine or its metabolites are believed to contribute to the disorder. The molecular pathways involved in the neuropathology in both disorders are ill defined. Here we show that a brief exposure to Mn increases reactive oxygen species, glutathione production, decreases oxygen consumption, head mitochondrial membrane potential, and confers DA neuronal cell death. DA neuron vulnerability is partially dependent on the DA neuron-expressing DMT-1 orthologue, SMF-1. PD associated neurotoxin-induced cell death is also partially dependent on SMF-1 as well as SMT-2, and DA neuron vulnerability in both models are inhibited by the *C. elegans* GST-pi orthologue, GST-1. We will also describe our studies that indicate specific ER stress response proteins play roles in the neuroprotection. We have also generated antibodies to over 40 *C. elegans* proteins, including PD-associated orthologues, and DA- and putative DAT-associated proteins, and we are analyzing protein expression and localization patterns under basal conditions and response to PD-relevant toxins. We will also describe our initial results from a novel genetic screen to identify modulators of PD- and manganese-associated DA neuron vulnerability. Support contributed by: NIH R01ES014459, NIH R01ES010563, W81XWH-05-1-0239 MHRR (RN).

### 1291 EXPOSURE TO GLYPHOSATE-CONTAINING HERBICIDES OR COMBINED TREATMENT WITH MANZATE LEADS TO SELECTIVE NEURODEGENERATION IN CAENORHABDITIS ELEGANS.


Parkinson's disease (PD), which results from loss of nigral dopaminergic (D1ergic) neurons, has been linked to high pesticide usage and exposure. In order to test the hypothesis that commonly-used pesticides may lead to neurodegeneration, we used the model organism *C. elegans*. Initially we treated wild-type (N2) worms with varying concentrations of two glyphosate-based herbicides: RoundUp (RU) and...
Manganese (Mn) is widely distributed in the atmosphere; although it is an essential metal, it has been reported that the overexposure causes neurotoxicity manifested as extrapyramidal symptoms similar to those observed in Parkinson disease (PD); some authors have proposed that these signs are related to the alteration of the globus pallidus and substantia nigra reticulata functions, however no experimental information is available about its effects on the structures directly involved in PD. For that reason, the goal of this study was to determine the effect of MnCl2-MnOAc3 mixture on substantia nigra compacta (SNc) and striatum through ultrastructural analysis. CD-1 (35g) male mice inhaled 0.02M AcMn3 and 0.04M MnCl2 one hour twice a week, during 40 weeks; then sacrificed and the SNc and striatum were processed for electron microscopy. The Mn exposed animals showed neuropil alterations, decrease of axo-synaptic synaptic contacts, increase of peroxidized synapses in the striatum and nuclear alterations, necrosis and apoptosis in both nuclei. These results provide evidence that Mn inhalation produces ultrastructural alterations similar to those observed in Parkinson disease.

1293 REDOX METABOLOMIC ANALYSIS OF PLASMA GLUTATHIONE REDOX STATUS OF HUMAN PARKINSON’S DISEASE.

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Oxidative stress plays a significant role in Parkinson’s disease development. Research from our laboratory utilizing redox metabolomic techniques has shown that oxidation of GSH/GSSG redox state correlates with aging, disease and toxic exposure. Alterations in thiol redox status (cysteine (Cys), cystine (CySS), GSH, GSSG, and Eh) using the established method were measured in plasma samples of human PD patients and compared to a healthy, aging cohort. Results show statistically significant increases in plasma concentrations of Cys and CySS in PD patients compared to the healthy controls. The plasma GSH concentration was unchanged due to PD; however, plasma GSSG was significantly elevated in PD patients. Increased plasma GSSG concentration resulted in a significant oxidation of the plasma GSH/GSSG redox state. These preliminary findings indicate PD-mediated oxidative stress may be measurable with this method. Characterization of elevated plasma Cys and CySS as potential biomarkers for PD will be discussed.

1294 SYNERGISTIC TOXICITY OF ARSENIC AND DOPAMINE IN SH-SY5Y NEUROBLASTOMA CELLS AND THE PROTECTIVE EFFECT OF NICOTINE.

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Parkinson’s disease (PD) is a neurodegenerative disorder that is characterized by decreases in the formation and effects of dopamine. Whereas the etiology of PD is currently unknown, one potential cellular mechanism may involve environmental toxins such as arsenic, an established neurotoxin that is a component of certain pesticides and which is an environmental contaminant. The present study was conducted to examine the synergistic effects of dopamine and arsenic in a tissue culture model employing human neuroblastoma cells (SH-SY5Y) and to determine if these effects of arsenic and dopamine when used alone and/or in combination may be ameliorated by nicotine, a proposed treatment for PD. When cells were incubated with a combination of dopamine (50μM) and varying levels of arsenic (2.5μM and 5μM), cell viability decreased significantly from control with a percent survival of 57% and 28.3%, respectively (p < 0.05). When nicotine was added to the cells, there was a significant decrease in the observed toxicity resulting from both arsenic and/or dopamine, when used alone or in combination. The protective effect was most significant at the highest levels of toxicity (arsenic 5μM, dopamine 100μM, nicotine 400 nM; p < .001). Results of this study indicate that arsenic and dopamine act in a synergistic toxic in SH-SY5Y neuroblastoma cells and that this toxicity may be attenuated by nicotine administration. These results support the existing hypothesis that the expression of Parkinson’s disease may be related to environmental toxins such as arsenic, and that nicotinic mechanisms may represent a potential direction for future therapy.
**1297 INVESTIGATION OF THE NEUROTOXIC MECHANISMS INVOLVED IN BETA-AMYLOID DEPOSITION IN PSAPP MICE.**

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Introduction: Amyloid-beta is endogenously formed neuronal peptide which has been proved to have a causal relationship with neurodegeneration in Alzheimer's disease (AD). MAP kinase, CREB, ERK and pCREB have played a vital role in memory regulation. The present study employed PSAPP mice expressing the 'Swedish' amyloid precursor protein and M146L presenilin-1 (PSAPP) mutations to study the cellular mechanisms and biomarkers involved in Aβ toxicity in relation to the loss of memory. Experimental Procedures: PSAPP mice and non-transgenic controls (eight months old) were subjected to behavioral and biochemical studies. Brains were dissected, hippocampus and cortex were removed. Behavioral experiments such as Y-maze and open field were performed along with Aβ deposition (1-40 and 1-42). Enzymatic activity of beta secretase as well as the alteration in the cellular signaling pathways (ERK MAP kinase, STAT and CREB pathways were analyzed by multiplex microbeads method. ANOVA and Dunnett's test were used to compare the results with non-transgenic mice. Results and Conclusion: This study reveals the alterations in behavioral and cellular processes that occur due to Aβ in PSAPP mice. A significant decrease in CREB and ERK expression was observed in the testes of rats exposed neonatally to BPA. The present study revealed the cellular mechanisms and biomarkers involved in Aβ toxicity in relation to the loss of memory.

**1298 NEONATAL EXPOSURE OF MALE RATS TO BISPHENOL A IMPAIRS EXPRESSION OF SERTOLI CELL JUNCTIONAL PROTEINS IN THE TESTIS.**

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Sertoli cell junctional proteins (SCJ) (viz. adhesion, gap and tight junctions) are important for spermatogenesis and perturbations in expression of these proteins are associated with impairments in process of sperm production. Bisphenol A (BPA) is an endocrine disrupter that has been associated with impaired spermatogenesis. However the mechanistic basis of impaired spermatogenesis is unknown, whether BPA is a Sertoli cell toxicant has not yet been fully investigated. The present study was undertaken to decipher the effects of neonatal exposure of male rats to BPA on the testicular expression of SCJ in developing male rat. Neonatal male rats were s.c. injected with 2.4 μg/kg bw of BPA in sesame oil from postnatal day 1-5 and controls received vehicle. Immunohistochemical localization for Connexin 43 (Cx-43, gap junctional), Zona Occludin-1 (ZO-1, tight junctions) and N-cadherin (adherens junction) was carried out on testicular tissue sections obtained from PNDs 15, 30, 45 and 90 of rats exposed to the lowest dose of BPA2.4 μg/kg/day that impaired fertility. A significant reduction in the expression of Cx-43 (PND 45 and 90) and increases in the expression of N-cadherin (PND 45 and 90) were observed in the testes of rats exposed neonatally to BPA. Interestingly, there was an altered expression pattern of Cx-43 amongst the sloughed cell in the testes of the experimental rats as compared to controls. Neonatal exposure of BPA to rats has the potential to induce perturbations in SCJP. These perturbations may be one of the contributing factors that lead to impairments in spermatogenesis in the exposed animals and can be used as potential biomarkers to study BPA-induced effects on testes.

**1299 DETECTING BIOMARKERS OF CHRONIC ARSENIC EXPOSURE BY USING SELDI-TOF-MS PROTEIN CHIP TECHNOLOGY.**

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Background: Chronic exposure to high levels of inorganic arsenic that is naturally present in drinking water in certain geographic regions has become a major public health concern in China. In this study we used surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) to determine if arsenic exposure in drinking water could induce serum protein changes and to identify new biomarkers for chronic arsenic exposure. Methods: A total of 120 subjects were selected in three groups based on their exposure levels in drinking water (3.2±2.5 μg/L, 22.1±4.7 μg/L, and 177.6±23.8 μg/L, respectively). Serum protein profiles were analyzed by SELDI-TOF-MS with a CM10 Protein Chip. Diagnostic model was constructed by decision tree algorithm in a training set with 120 subjects and validated in a testing set with other 58 subjects. Results: Relative intensities of 41 protein peaks were found differentially among three groups. A panel of five proteins with mass-to-charge ratio (m/z) of 287.48, 612.41, 7580.58, 9432.56 and 5552.66 was selected to build the diagnostic model. Among these markers, the 287.48 Da and the 7580.58 Da were significantly up-regulated or down-regulated only in the group of subjects exposed to 177.6±23.8 μg/L of arsenic. The 612.41 Da and the 5552.66 Da were significantly down-regulated in the groups with arsenic exposure levels of 22.1±4.7 μg/L and 177.6±23.8 μg/L. The 9432.56 Da content was the lowest in the group exposed to 22.1±4.7 μg/L of arsenic. The power to detect differences among three groups in the testing set was evaluated with the sensitivity of 80.00%-86.67%, and the specificity of 86.67%-95.35%. Conclusion: Exposure to 22.1±4.7 μg/L of arsenic in drinking water is enough to cause changes in serum protein profiles. This proteomic technology showed very promising in detecting levels of arsenic exposure and discovering new biomarkers.

**1300 EFFECTS OF ORAL ADMINISTRATION OF PILOGLITAZONE, SODIUM SACCHARIN OR SODIUM O-PHENYLENEDIAMINE ON THE EXPRESSION OF ONCOMODULIN IN THE BLADDER EPITHELIUM OF MALE F344 RATS.**

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Currently there are no reliable markers for early detection of urinary bladder cancer. Recent studies have shown that expression of the oncomodulin gene is increased in urothelium from rats treated with various bladder carcinogens. Pioglitazone is a PPARγ agonist which induces rat bladder tumors. We administered pioglitazone in 0.5% methylcellulose (MC) intragastrically (i.g.) to evaluate the level of oncomodulin expression in F344 rat urinary bladder epithelium. Sixty male F344 rats were randomly divided into 4 groups of 15 rats each and treated for 4 weeks with: 1) control diet and daily MC i.g.; 2) control diet and 16 mg/kg pioglitazone in MC i.g.; 3) diet containing 7.5% sodium saccharin (NaSac) and MC i.g.; or 4) diet containing 2.0% sodium o-phenylphenate (NaOPP) and MC i.g. RT-PCR was employed to detect expression of oncomodulin in the urothelium. Light microscopy, SEM, and immunohistochemical detection of BrdU were used to examine cytotoxic and proliferative urothelial effects. Expression of oncomodulin was significantly increased in NaSac or NaOPP-treated groups compared to controls, but not in the pioglitazone group. All test chemicals induced superficial necrosis by SEM and increased BrdU labeling index indicative of increased cell proliferation. In vitro, PPARγ agonists induced differentiation of U2OS osteoblast-like, was down-regulated, decreased proliferation, and decreased oncomodulin expression. Unlike NaSac and NaOPP, pioglitazone did not induce an increase in oncomodulin expression, possibly related to its competing effects of: 1) indirectly increasing urothelial proliferation by inducing production of urinary solids, and 2) decreasing proliferation due to direct effects on urothelial PPARγ.

**1301 SIMULTANEOUS ANALYSIS OF ELEVEN VOC METABOLITES IN HUMAN URINE.**

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Sponsor: B. Fowler

Volatile organic compounds (VOCs) are ubiquitous in the environment, originating from many different natural and anthropogenic resources, including tobacco smoke. Long-term exposure to certain VOCs may increase the risk for cancer, birth defects, and neurocognitive impairment. Therefore, VOC exposure is an area of significant public health concern. We developed a reversed-phase high-performance liquid chromatography coupled with electro-spray ionization tandem mass spectrometry (LC-ESI/MSMS) method to quantify urinary VOC metabolites as biomarkers of exposure. In the current method we monitor N-acetyl-S-(2-hydroxyethyl)-L-cysteine (HEMA), N-acetyl-S-(3-hydroxypropyl)-L-cysteine (HPMA),...
S-(1-hydroxy-3-buten-2-yl)-N-Acetyl-L-cysteine (MHBMA), N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (DHBMA), N-Acetyl-S-(2-carboxyethyl)-L-cysteine (CMA), and N-Acetyl-S-(2-phenyl)-L-cysteine (PMA), N-Acetyl-S-(benzyl)-L-cysteine (BMA), 2-thiobis(hydroxymethyl)-2-carboxylic acid (TTCA), N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine (AMCC), N-Acetyl-S-(2-carboxymethyl)-L-cysteine (AAMA) and N-Acetyl-S-(trichlorovinyl)-L-cysteine (TCVMA) in human urine. These analytes are metabolites of 1,3-butadiene (MHBMA, DHBMA), benzene (PMA), toluene (BMA), acrylamide (AAMA), carbon disulfide (TTCA), N,N-dimethylacetamide (AMCC), N-acetyl-S-(benzyl)-L-cysteine (TCA), N-acetyl-S-(glycylcarbamoyl)-L-cysteine (TCVMA), acrylonitrile, vinyl chloride, and ethylene oxide (HEMA). For matrix spiked experiments the mean accuracy ranges from 98-107% and the mean percent difference ranges from 0.43-9.54%. The limit of detection ranges from 0.01-21 µg/L. By spiking urine with pure isomers and retention time interpretation, we could identify the correct diastereomer of MBHMA in human urine as S-(1-hydroxy-3-buten-2-yl)-N-Acetyl-L-cysteine. We applied this method to 690 urine samples collected (10 samples each) from 25 smokers and 44 non-smokers (categorized based on blood 2.5-dimethylurifan levels) to find that smokers have significantly elevated levels of AAMA, CEMA, DHBMA, HEMA, HPMA and PMA.

1302 TOXICOGENOMIC IDENTIFICATION OF BIOMARKERS OF ACUTE RESPIRATORY EXPOSURE TO SENSITIZING AGENTS.
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Allergy induction requires multiple exposures to an agent. Therefore the development of high-throughput or in vitro assays for effective screening of potential sensitizers will require the identification of biomarkers. The goal of this preliminary study was to identify potential biomarkers that differentiate the response to allergen vs non-allergen agents using an acute exposure model. Female BALB/c mice received a single intratracheal aspiration exposure to Metrizaimis aniloplaize crude antigen (MACA) or bovine serum albumin (BSA) in Hank’s Balanced Salt Solution (HBSS) or HBSS alone. Mice were sacrificed after 1, 3, 6, 12, 18 and 24h. Bronchoalveolar lavage fluid (BALF) was evaluated to determine total and differential cellularity, total protein concentration and LDH activity. RNA was isolated from lung tissue for microarray analysis and RT-PCR. MACA administration induced a rapid increase in BALF neutrophils, lymphocytes, eosinophils and total protein levels as compared to BSA or HBSS. Microarray analysis demonstrated differential expression of genes involved in cytokine production, signaling, inflammatory cell recruitment, adhesion and activation in 3h and 12h MACA-treated samples as compared to BSA or HBSS. Further statistical and pathway analyses allowed identification of ~100 candidate biomarker genes. Eleven genes were selected for further assessment by qRT-PCR. Of these, 6 demonstrated increased expression (Ccl17, Ccl22, Ccl7, Cxcl10, Cxcl2, Saa1), while C3ar1 increased from 6-24h. In conclusion, a single respiratory exposure of mice to an allergenic mold extracts induce an inflammatory response which is distinct in lungs from rat liver using intra-venous injection of clodronate (CLO) liposomes. KC depletion or depletion of KCs may also contribute to serum enzyme elevation. In order to assess the possibility of tissue injury. For example, an increase in the serum level of the enzyme alanine aminotransferase (ALT) is a sensitive indicator of hepatic injury. However, changes in steady state levels of certain in vivo enzymes do not reflect a change in either their rate of release to the bloodstream or their clearance from the bloodstream. Since the turnover of many serum enzymes occurs via receptor mediated endocytosis by Kupffer cells (KC’s) in the liver, it is possible that inhibition of Kupffer cells (KCs) may also contribute to serum enzyme elevation. In order to better understand the role of KCs in serum enzyme clearance, KCs were depleted from rat liver using intra-venous injection of clodronate (CLO) liposomes. KC depletion was monitored using immunohistochemistry with antibodies to ED1 and ED2, which detect immature and mature (ED1) or just mature (ED2) KCs. ED2-positive cells were undetectable at 24 hours, with repopulation evident at 72 hours, and near to baseline levels by 9 days post CLO administration. The serum levels of ALT, aspartate aminotransferase (AST), creatine kinase (CK), glutamate dehydrogenase (GLDH), and lactate dehydrogenase (LDH) ALT were measured at 4, 8, 24, 48, 72, 96 hours, and 8 days post administration of CLO liposomes. The maximal increase was 8x at 8 hours for CK, and 4x, 10x, and 25x at 24 hours for AST, GLDH, and LDH, respectively with minimal changes in ALT. The increases in serum enzymes were inversely correlated to decreases in levels of KCs and returned to baseline by day 8. Histopathology of liver, heart, and skeletal muscle was normal and no changes to tropism 1 were noted, suggesting that CLO administration did not cause direct injury to these tissues. These data further demonstrate the role of KCs in serum enzyme clearance and support another mechanism for serum enzyme elevation that is not related to liver or muscle injury.
1306 DEVELOPMENT OF AN IN VITRO ASSAY FOR RESPIRATORY SENSITIZATION CONSIDERING THE VISION ON TOXICITY TESTING IN THE 21ST CENTURY.

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Despite regulatory requirements there is no established protocol for the identification of chemical respiratory sensitizers. New tests should be based on mechanistic understanding and should be preferentially restricted to in vitro assays. To contribute to the development of a respiratory sensitization assay considering the vision on toxicity testing in the 21st century, we performed transcriptomics experiments and studied alterations in gene expression in a BEAS-2B lung model after exposure to the respiratory sensitizers ammonium hexachloroplatinate IV, hexamethylene diisocyanate, and trimellitic anhydride, the irritants crocetin and methyl salicylate, and the skin sensitizer 1-chloro-2,4-dinitrobenzene. Agilent Whole Human Genome arrays used in this study revealed markers that are able to discriminate respiratory sensitizers from respiratory non-sensitizers. One of the interesting genes is CASP9, which is known to be associated with asthma and/or respiratory sensitization. When categorizing the 1000 most discriminative genes into biological Gene Ontology terms, 20 genes were associated with immune function. The majority of these genes have already been studied in the context of airway inflammation, asthma, and respiratory sensitization (e.g. CCL24, CEBPB, MIF, and TLR4). We also hypothesized on possible toxicity pathways that could be associated with respiratory sensitization based on pathway analysis. Within this cellular model the phosphatase and tensin homolog (PTEN) pathway was identified as possibly specific for respiratory sensitization. These preliminary transcriptomics data add a new aspect to the action in the field to develop an in vitro respiratory sensitization assay, taking into account the new vision on mechanism-based toxicity testing.

1307 SELECTIVE MEASUREMENT OF ALT ISOFORMS IN RAT SERUM BY LC/MS.

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Alanine aminotransferase (ALT) activity in serum has long been used as a bio-marker of hepatotoxicity in clinical and preclinical studies and increases often correlate with observed histopathological findings in the liver. Occasionally, increases in serum ALT have been measured with no concurrent liver histopathological findings in preclinical species and can compromise the safety profile of a drug candidate. To date, there are two known forms of ALT which are differentially expressed in tissues and subcellular compartments and thus may serve as distinct markers for liver injury. The isozymes are expressed differently in various organs. Sequence analysis provided the sequences of canine ALT-1 and ALT-2 which are expressed differently in the various organs. But the protein levels determined by LC/MS have been compared with ALT activity levels measured by other assays. This data is being used to understand the role of each ALT isozyme in disease progression in the rat.

1308 SERUM CARDIAC TROPONIN I CONCENTRATIONS ARE TRANSIENTLY INCREASED IN RATS DOSED WITH ROSIGLITAZONE, A PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ AGONIST.

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Serum alanine aminotransferase (ALT, also known as glutamate pyruvate transaminase, GPT) is widely used as a marker for hepatic damage in clinical and non-clinical practice. However, it is thought that ALT elevation in the serum could be caused by effects on other organs other than only due to hepatic damage. In recent years, it has been shown in humans, mice and rats that ALT has two isoforms (ALT1 and ALT2) which are expressed differentially in the various organs. Serum ALT activity in rats after 168 hours of dosing at 80 mg/kg/day, returning to 2 pg/mL after 336 hours of dosing underscoring the temporal nature of cTnI increases. This is the first study detecting serum cTnI increases in rats administered rosiglitazone. This effect may be linked to functional changes because rosiglitazone-induced myocardial hypertrophy is postulated to be the result of positive inotropic and lusitropic effects without changes in heart rate, ventricular pressures and hematocrit. In light of reported cardiac events in patients chronically dosed with PPARγ agonists, our results support cTnI as the earliest biomarker heretofore of cardiac liability associated with these compounds.

1309 DISTRIBUTION ANALYSIS OF THE ALT ISOZYMES IN CANINE TISSUES.

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Serum alanine aminotransferase (ALT, also known as glutamate pyruvate transaminase, GPT) is widely used as a marker for hepatic damage in clinical and non-clinical practice. However, it is thought that ALT elevation in the serum could be caused by effects on other organs other than only due to hepatic damage. In recent years, it has been shown in humans, mice and rats that ALT has two isoforms (ALT1 and ALT2) which are expressed differentially in the various organs. But the isoforms in dogs, which are commonly employed in drug development, are not well studied. To comprehend the non-clinical data more precisely, in the present study we investigated the sequences of the canine ALT isoforms and their expression in various organs. Sequence analysis provided the sequences of canine ALT1 and ALT2, which are highly preserved in the other mammals. Based on the sequences, gene expression levels in the organs were analyzed by quantitative real-time PCR and specific polyclonal antibodies induced in rabbits, and then the expression of the isoforms was analyzed by western blotting and immunohistochemistry. These analyses showed that the isoforms are expressed differently in an organ-dependent manner; ALT2 is plentifully expressed in muscle, adipose tissue and kidney cortex while ALT1 is dominant in liver, heart and gastric mucosa. In addition, immunohistochemistry demonstrated characteristic distribution in the canine organs or cells. Strong ALT1 immunoreactivity was noted in the hepatocytes, myocardiacides, parietal cells, etc, while ALT2 reactivity was seen in the proximal renal tubules, striated muscle, neuronal cell bodies, etc. The present findings could be of great value for better understanding of serum ALT elevation in canines, especially in the case of absence of histopathological changes in the liver.

1310 CYTOKINES OF INFLAMMATION AND OXIDATIVE STRESS IN THE URINE OF COCAINE USERS.

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Cocaine is a powerful sympathomimetic associated with systemic inflammation, oxidative stress and vascular dysfunction. Its use has been linked to renal disease, rhabdomyolysis, vasoconstriction, acute myocardial ischemia and infarction. Systemic vascular damage has been linked to the induction of inflammatory and oxidative stress pathways. This study examines the expression of inflammatory, stress and vascular biomarkers in the urine of cocaine users. Commercially available ELISA test kits were used to assay urine specimens for markers representative of these pathways, including several interleukins, myeloperoxidase, high sensitivity c reactive protein, myoglobin, prostatic natriuretic peptide, creatin kinase MB isoenzyme, aldosterone, microalbumin, neutrophil gelatinase associated lipocalin, vascular endothelial growth factor and heat shock protein 90. Several of these have not yet been validated in urine; however, urine specimens are non-invasive and could prove useful for qualitative diagnostic testing once appropriate reference ranges have been identified. Increased expression of these markers is associated with better characterization of its cardiac effects and determine if cardiac troponin I (cTnI) can serve as an early biomarker for cardiac liability, male Wistar rats were orally administered 0, 10 or 80 mg/kg/day rosiglitazone. Myocardial gene expression profiling, histopathology, histology and clinical chemistry, including measurement of cTnI concentrations with the Singulex Erenna® Ultrasensitive Immunoassay, were evaluated after 6, 24, 168 and 336 hours of dosing. Heart weight mildly increased after 168 (~10%) and 336 (~15%) hours of dosing at 80 mg/kg/day in the absence of microscopic changes. At the transcriptomics level, gene categories typically associated with myocardial damage were not over-represented. Most importantly, cTnI transiently increased from 2.2±4 pg/mL in vehicle-treated rats to 19±4 pg/mL in 5/9 rats after 168 hours of dosing at 80 mg/kg/day, returning to 3±2 pg/mL after 336 hours of dosing underscoring the temporal nature of cTnI increases. This is the first study detecting serum cTnI increases in rats administered rosiglitazone. This effect may be linked to functional changes because rosiglitazone-induced myocardial hypertrophy is postulated to be the result of positive inotropic and lusitropic effects without changes in heart rate, ventricular pressures and hematocrit. In light of reported cardiac events in patients chronically dosed with PPARγ agonists, our results support cTnI as the earliest biomarker heretofore of cardiac liability associated with these compounds.
vascular damage, oxidative stress and inflammation. Establishing a link between biomarker expression in urine and cocaine-induced inflammation or cardiac damage is the first step in developing a diagnostic tool or assay panel that may permit rapid diagnosis and selection of an appropriate clinical intervention. Piggybacking assays for markers of oxidative and inflammatory changes, particularly those associated with cardiac damage, alongside the illicit drug panel might allow clinicians to identify patients at risk of renal or vascular events. Our research suggests that significant differences exist in the urinary expression of certain biomarkers and that this may be an important non-invasive way to assess oxidative, vascular and inflammatory damage in users of illicit substances. This work has been supported in part by the Agency for Community Treatment and Services of Tampa.

1313 EVALUATION OF URINARY BIOMARKERS IN EARLY STAGE OF KIDNEY INJURY INDUCED BY AMINOGlyCOSIDES.

Background: For decades, a histopathological examination and blood test have been mainly used to evaluate renal toxicity in animal studies. However, these are able to detect only progressed kidney injury. In this study, we aimed to explore urinary biomarkers, which are related to early kidney injury being not associated with functional failure and allow us to provide better management of kidney injury in clinical site. FDA and EMEA have recently introduced new urinary biomarkers of kidney injury. We examined the changes in new urinary biomarkers as well as traditional blood and urine biomarkers in early stage of kidney injuries induced by aminoglycosides. Materials and methods: Male F344/DaGrl (Fischer) rats were intramuscularly treated for 9 days with gentamicin (GM: 10-80 mg/kg/day), tobramycin (TOB: 20-80 mg/kg/day), amikacin (AMK: 80 mg/kg/day) or amikacin (AMK: 80 mg/kg/day). We conducted urinary tests (Day 3, 6 and 9), blood tests (Day 10) and histopathological examination (Day 10). Results: GM, TOB and ABK increased almost all urinary biomarkers, but some of the markers were not increased by TOB or ABK. AMK did not increase any urinary biomarkers. In blood tests, these drugs did not change BUN, GM, TOB and ABK induced pyknosis, eosinophilic granule and/or basophilic changes in the tubular epithelium, and AMK induced only eosinophilic granule in the tubular epithelium. These findings were considered to be marginal changes without toxicological significance. In the ROC analysis, pyknosis was well correlated with NAG, beta2-microglobulin, clusterin, GST-Yb1 and VEGF. Discussion: GM, TOB and ABK induced pyknosis, which is known as an early histopathological change in kidney injury, but did not induce functional changes. Some urinary biomarkers were well correlated with pyknosis. It was suggested that the urinary biomarkers were useful to detect early kidney injury. These biomarkers are expected to help the management of kidney damage in clinical site.

1314 EVALUATION OF GENDER AS A VARIABLE IN RENAL BIOMARKER RESPONSE IN RATS GIVEN A NEPHROTOXIC DOSE OF GENTAMICIN.
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The majority of studies to evaluate renal safety biomarkers have used male rats. This study was conducted in male (M) and female (F) rats to evaluate potential gender differences in baseline and induced levels of nephrotoxic biomarkers in response to gentamicin treatment. Adult Sprague-Dawley rats given daily subcutaneous injections of 75 mg/kg/day gentamicin were euthanized on Day 4 or 11. Blood samples were collected at necropsy and 16-hour urine samples were collected pre-dosing and before necropsy. Urinary parameters evaluated (after normalization to UCr) included NAG, microalbumin, RPA-1, β2-microglobulin, calbindin, clusterin, cystatin C, EGF, GST-α, GST-Yb1, KIM-1, NGAL, osteopontin, TIMP-1 and VEGF. Kidney microscopic findings on Day 4 were limited to minimal hyaline droplet formation in proximal tubules (PTs) in all treated M, while on Day 11 the observed changes were generally marked and similar in both sexes (degeneration/necrosis of PTs and regeneration/basophilia of proximal and distal tubules and of cortical collecting ducts). Findings on Day 11 were associated with increases in BUN and SCr in both sexes (between 3.0 and 3.6 fold). There were statistically significant differences between M and F in baseline values of some analytes, particularly β2-microglobulin (higher values in M than in F). On Day 4, in treated M and F there were similar increases in GST-α (2.4 and 3.8-fold), β2-microglobulin (4.4 and 2.3-fold), NGAL (2.6 and 2.5-fold) and calbindin (3.9 and 5.5-fold). On Day 11, marked increases (higher than 10-fold) were noted in both sexes for most novel biomarkers evaluated, except for EGF that was decreased in M and F (9.3 and 3.8-fold, respectively). Thus, although there were gender differences in baseline levels of some biomarkers, changes induced following gentamicin treatment were of similar magnitude in both sexes. This supports the translation to F rats of biomarker qualification studies that were conducted in M rats.
1315 EVALUATION OF 3- AND 1-METHYLHISTIDINE AS PRECLINICAL URINE BIOMARKERS OF DRUG-INDUCED SKELETAL MUSCLE TOXICITY IN RATS.

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Methylation of histidine in the histidine ring occurs at position 3 (actin and myosin) or position 1 (serine) and the urinary excretion of 3-MH is used as an index of muscle protein breakdown. The purpose of this work was to evaluate the utility of 3- and 1-MH as urine biomarkers of drug-induced skeletal muscle injury in the female rat. Both 3- and 1-MH were measured by GC/MS, and a broad tissue comparison indicated that skeletal muscles contained approximately 49 and 218 umol/g of 3- and 1-MH, respectively, with no difference in fast- and slow-twitch muscles. These concentrations were at least 50-times higher than other tissues, indicating that 3- and 1-MH are skeletal muscle specific. Changes in 3- and 1-MH were then evaluated against histopathology and other serum biomarkers including fatty acid binding protein 3 (Fabp3), skeletal troponin I (sTnI) and myosin light chain 3 (Myl3) in a model of cerivastatin (1 mg/kg/day)-induced myopathy. The concentrations of Fabp3, sTnI and Myl3 were generally below the limit of detection (<1 ng/ml). Following 10 doses of cerivastatin, a minimal myopathy was observed histologically and urine 3- and 1-MH increased by almost 2-fold, results that were consistent with increased serum concentrations of Fabp3, sTnI and Myl3. Severe myopathy developed after 14 doses of cerivastatin and was accompanied by a greater than 2- and 10-fold increase in urine 3- and 1-MH, respectively. These changes were again consistent with increased serum concentrations of Fabp3, sTnI and Myl3. Collectively, these data indicate that 3- and 1-MH are highly specific to skeletal muscle and may represent useful urine biomarkers of drug-induced myopathy in rats.

1316 IN VITRO HIGH CONTENT SCREENING OF CIGARETTE SMOKE MEDIATED BIOMARKERS OF EFFECT: TOBACCO TYPES.


The aim of this study was to identify biomarkers of effect associated with cancer, COPD and cardiovascular effects of tobacco smoke and also differentiate between tobacco types for their comparative effects on these biomarkers. Commercial cigarettes contain a blend of tobacco types/grades that produce varying levels of 471 volatile constituents. Therefore, these tobacco types can differentially induce biomarkers in vitro. We evaluated the effects of smoke particulate matter collected from Burley (upper stalk), Flue Cured (lower stalk), and Oriental (mid-upper stalk) tobaccos on neutral red uptake (NRU), 8-iso-prostaglandin F2α (8-iso-PG2α), mitochondrial membrane potential, cell permeability, caspase-3, nuclear size, H2AX-phosphorylation, interleukin-8 (IL-8), and heme oxygenase-1 (HO-1) in human lung A549 cells by ELISA and multiparameter high content screening. All measured biomarkers exhibited dose-dependent changes in smoke exposed A549 cells. Mitochondrial membrane potential (early apoptosis biomarker), H2AX-phosphorylation (DNA double-strand break biomarker), 8-iso-PG2α (lipid peroxidation biomarker), and cell permeability all showed dose-dependent increases with no significant differences (p>0.05) between tobacco types. Burley tobacco had less adverse effects (p<0.05 on NRU (cytotoxicity), HO-1 (protection against oxidative stress), cell number and, caspase-3 (apoptosis biomarker) when compared to Flue Cured and Oriental tobaccos. However, Burley tobacco induced significantly higher levels of IL-8 (inflammation biomarker) than Flue Cured and Oriental tobaccos (p<0.05). From this study, five biomarkers: NRU, cell number, IL-8, mitochondrial membrane potential, and HO-1, were successful in discriminating toxicity of one or more of the tobacco types from the others.

1317 SERUM CYTOKERATIN 18 AND ADIPOCYTOKINES IN ALCOHOLIC HEPATITIS VERSUS NON-ALCOHOLIC STEATOHEPATITIS.

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Alcoholic steatohepatitis (ASH) and nonalcoholic steatohepatitis (NASH) have similar pathologic appearances, but biomarkers and mechanisms may be different. The purpose of the study is to examine these issues in human subjects. From the UofL Liver Disease Specimen Bank, we obtained archived fasting serum samples from human subjects with either ASH or NASH as well as healthy controls with no known history of alcohol abuse. Cytokereatin 18 (CK18), adipocytokines, and antioxidants were determined. CK18 whole and caspase-cleaved fragment and biomarkers of necrosis and apoptosis, were higher in ASH than in NASH. Inulin was similarly elevated in both ASH and NASH, but adiponectin was low in NASH, whereas it was elevated in ASH. Leptin was high in NASH, reflective of obesity but it was low in ASH. Pro-inflammatory cytokines including TNF-alpha, IL-1, IL-6, and IL-8 were normal in NASH but were elevated in ASH. Antioxidants were reduced to a greater degree in ASH than NASH. Multiple differences exist between ASH and NASH. The key findings of the study were that the magnitude of CK18 dysregulation was markedly greater in ASH, probably reflective of disease severity. Although hyperinsulinemia was present in both ASH and NASH, adiponectin levels were divergent.

1318 CORRELATION OF URINARY BIS(MONOAICYL)GLYCEROL PHOSPHATE LEVELS WITH DRUG-INDUCED PHOSPHOLIPIDOSIS IN THE RAT.

K. Thompson, K. Haskins, B. Rosenzweig, S. Stewart, D. Peters, R. S. Pine and J. Hang. DARP, CDER, U.S. FDA, Silver Spring, MD.

Phospholipidosis is a lipid storage disorder characterized by the excessive accumulation of phospholipid in intracellular vesicles. This effect has been produced in preclinical studies by more than 250 approved or investigational drugs but its incidence in the clinic is uncertain due to the lack of sensitive biomarkers that can be measured in accessible biofluids. One proposed non-invasive biomarker of phospholipidosis is the rare phospholipid bis(monoacyl)glycerol phosphate (BMP), which is an enriched component of endosomes. Increased levels of BMP have been observed in serum, tissues, and urine in a limited number of studies of animal models of drug-induced phospholipidosis. To further characterize its performance, urinary BMP levels were measured in rats that were treated with the known phospholipidotopic drugs amiodarone, azithromycin, cilastrol, desloratadine, or maprotolite for 2-4 weeks. An elevation in urinary BMP normalized to creatinine was seen in all treatment groups that had an increased numbers of alveolar macrophages characterized by vaculated cytoplasm or vacuolization in bile duct epithelium. Phospholipidosis identified by light microscopy was verified by electron microscopy or by immunostaining of tissue sections for LAMP2, a major surface protein of lysosomes that has been used to distinguish phospholipids encapsulated in late endosomal compartments from other lipid vacuoles. These results suggest that urinary BMP warrants further investigation as a potential non-invasive biomarker of phospholipidosis.

1319 MITOCHONDRIAL PATHOLOGIES INDUCED BY COMMON CHEMICALS AND TOXINS AS VALUABLE RESEARCH TOOLS.

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Mitochondria can supply 90% of the energy in many tissues and cell types, thus vital to normal organism function. Mitochondria (mito.) are pivotal control points in apoptosis and necrosis pathways, plus contain crucial metabolic processes. Common chemicals (medications, solvents, heavy metals, pesticides, etc.) can have adverse affects on the mito., directly or indirectly. Mitochondrial experimentation is complex and difficult to interpret, however, examples will be clarified how common chemicals/pesticides can inhibit vital mito. energy-production pathways (i.e. Complex I, II, or III, cytochrome oxidase, adenine nucleotide translocate, ATP synthase, etc.). For instance, Complex I inhibitors are correlated to Parkinson Disease development. Mitochondria have their own DNA and protein synthesis machinery, which can be adversely affected by these chemicals. Compounds can be respiratory uncouplers or inhibit aldehyde dehydrogenase—allowing the build up of endogenous toxic species (i.e. hydroxynonenal or acetaldehyde from ethanol metabolism). Chemicals may induce mito. swelling leading to necrosis, or inhibit apoptosis leading to tumorgenesis. Important mito. pathways affected by these compounds can result in cellular or tissue dysfunction (i.e. fatty acid metabolism, steroid synthesis, calcium homeostasis, citric acid cycle, monoamine oxidation, urea cycle, etc). Chemicals which stress the lysosomes/autophagy or ER can result in mito. dysfunction due to calcium overload, drug metabolism/sequestration alterations, protein mis-folding, pathological signaling, or lipid/carbohydrate processing errors. The mito. matrix pH is alkaline, so amines can have very different properties there. Conversely, important examples will be presented how toxins are also valuable tools for medical research, to define mechanisms for pathology models. Mitochondria can occupy 20% of the cell volume and 40% of the protein content...
in some cells, thus considerably important targets of common chemical binding and action. The views expressed are not necessarily the policy or position of the U.S. EPA.

**PS 1320** SERUM CYTOKERATIN-18 LEVELS ARE NORMAL IN HIGHLY-EXPOSED STYRENE WORKERS SUGGESTING THAT THEY DO NOT SUFFER FROM LIVER NECROSIS OR APOPTOSIS.

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Hepatocellular necrosis is a typical finding in hepatitis due to industrial chemicals. Interestingly, routine liver enzymes are frequently normal in this setting, and a more sensitive biomarker is required. In our previous studies, serum whole cytokeratin 18 (neocartilage biomarker) was elevated in chemical workers with steatohepatitis due to vinyl chloride. These workers had normal routine serum liver enzymes (AST and ALT) as well as normal serum caspase-cleaved cytokeratin 18 (apoptosis biomarker). Controversy exists in the literature with regard to the potential human hepatotoxicity of styrene. The purpose of this study was to determine if cytokeratin-18 is elevated in highly-exposed styrene workers. We measured liver enzymes and cytokeratin 18 whole and caspase-cleaved fragments in the serum of highly-exposed styrene workers and healthy unexposed controls. Liver enzymes as well as cytokeratin 18 levels were similar to controls in these highly-exposed styrene workers. Highly-exposed styrene workers have normal cytokeratin 18 levels suggesting they are free from liver disease due to either hepatic necrosis or apoptosis.

**PS 1321** BIOMARKERS FOR HEPATIC HEMANGIOSARCOMA.

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Hepatic hemangiosarcoma (HH) is an extremely aggressive form of liver cancer which has historically been linked to high-level occupational exposure to vinyl chloride (VC). This association was first reported in 1974 in workers from a Louisville, KY plastics plant. To date, 26 VC workers have developed HH from this plant, and liver chemistries were surprisingly normal at diagnosis. Because of a long latency period, many workers are still at risk for the development of this deadly tumor and biomarkers are needed. The sinusoidal endothelial cell (SEC) is the progenitor for HH. SECs metabolize hyaluronidase (HA), a glycosaminoglycan component of the extracellular matrix. HA appears to play a role in carcinogenesis and is also a tumor marker for head and neck cancer. For these reasons we hypothesize that serum HA is a biomarker for HH. Serum HA was measured in VC workers with HH and in healthy controls, using archived samples in our specimen bank. HA was markedly elevated in VC workers with HH. As expected, routine liver enzymes were normal in HH and also in the healthy controls. HA appears to be a biomarker for vinyl chloride-related hemangiosarcoma. The applicability of these findings to other etiologies of hemangiosarcoma such as prescription medications (observed in rodents) should be explored.

**PS 1322** ANALYSIS OF KIDNEY DAMAGE BIOMARKERS IN PLASMA AND URINE SAMPLES FROM PATIENTS WITH DOCUMENTED RENAL INJURY.

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The kidney is one of the primary sites of xenobiotic-induced toxicity, which underlines the need for reliable and sensitive biomarkers for renal injury. The FDA and EMEA have issued guidelines for new urinary biomarkers of drug-induced kidney damage in rats, and a natural continuation to build on such efforts are studies performed in human patient material to advance the “rolling qualification”. To this end, a screen for potential protein biomarkers in relation to kidney toxicity/damage was performed in a set of urine and plasma samples from patients with documented renal damage. The investigated patient groups included diabetic nephropathy, obstructive uropathy, analgesic abuse and glomerulonephritis along with age and gender matched control groups. Multiplexed immunoassays were applied in order to quantify the following protein analytes: Alpha-1 Microglobulin, KIM-1, Microalbumin, Beta-2-Microglobulin, Calbindin, Clusterin, Cystatin C, Trefoil Factor-3, CTGF, GST- alpha, VEGF, Calbindin, Osteopontin, Tamm-Horsfall Protein, Temp-1, NGAL. In addition, standard blood and urine chemistry were determined. Statistical analyses using both univariate and multivariate tools indicated discrepancies in protein levels when comparing case and control samples. Future analysis aiming to qualify these biomarkers will indicate the potential of these candidates as markers for renal toxicity in humans.

**PS 1323** NOVEL BIOMARKERS OF CISPLATIN-INDUCED KIDNEY DAMAGE.

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Introduction – In 2008, the FDA and EMEA issued a joint framework allowing for the biomarker qualification and regulatory endorsement of 7 new urinary biomarkers of drug-induced kidney injury in rats. We sought to screen for other novel biomarkers of drug-induced kidney injury by utilizing the original Novartis study samples that were the source for the Predictive Safety Testing Consortium’s (PSTC) submission to the FDA and EMEA.

Experimental Procedures – Control and Cisplatin study samples with previously documented proximal tubular injury were utilized as screening study to select the most promising biomarker candidates. Nine further studies with compounds inducing tubular, glomerular and collecting duct injury are used as validation set.

Results – Several biomarkers significantly increased in urine and plasma which are linked to inflammatory pathways correlated to histopathological kidney damage severity. In addition, ROC analysis indicated that several of the novel analytes are sensitive and specific markers of drug-induced kidney damage with a significantly higher AUC of ROC than BUN and serum Creatinine.

Conclusions – This newly identified biomarkers have potential to complement the already known and partially qualified biomarkers for drug induced kidney injury in particular for monitoring inflammatory processes, whereas the 7 endorsed biomarkers are mechanistically linked to functional changes, cell injury and regeneration processes.

**PS 1324** ELECTROENCEPHALOGRAPHIC SIGNATURES PREDICT SEIZUREGENIC POTENTIAL OF NMDA RECEPTOR ANTAGONISTS KETAMINE AND MEMANTINE.

AstraZeneca Pharmaceuticals, Wilmington, DE.

Interictal spikes and/or spike-wave on the electroencephalogram (EEG) trace are recognized as signs of altered seizure threshold or seizure. However, very little is known about frequency changes on the EEG that precede ictal events, some of which may help predict the seizuregenic potential of a given chemical entity. We examined changes in EEG frequency elicited by NMDA channel blockers memantine and ketamine in C57BL6 mice. Memantine (50 mg/kg i.p.) causes generalized seizures in mice, while ketamine does not at any dose. Continuous EEG recordings were obtained from mice with electrodes implanted above motor and occipital cortices. Spectrograms were generated with fast Fourier analysis to visualize frequency power changes ranging from delta to gamma (1 to 80 Hz). Ketamine caused a significant power increase in the beta-gamma range by 38, 145 and 262% above vehicle controls at doses of 30, 60 and 120 mg/kg respectively. Ketamine dose at 60 and 120 mg/kg also elicited increases 200% above control in lower frequencies bands delta and theta. Memantine caused a beta-gamma power increase by 14, 214 and 252% above control at doses of 5, 25 and 50 mg/kg respectively. Frequent interictal spikes were seen in the EGs of animals dosed with 25 mg/kg memantine, while 50 mg/kg caused convulsions accompanied by spike-wave. Interestingly, memantine failed to elicit changes in lower frequency bands. We speculate that large increases in beta-gamma power in memantine-treated mice are associated with neuronal hyperactivity leading to seizures. Similar power increases in high frequency bands.
prior to kainic acid-induced seizures were recently reported (Bragin et al., 2009). In contrast, increases in lower frequency bands delta-theta, which are typically elicited by many anticonvulsants, may prevent generalized seizures from occurring following ketamine treatment. Our data suggest quantitative EEG analysis is a promising approach to assess the epileptogenic potential of CNS active compounds.

The systemic effects of obesity on the immune system are poorly understood, but often associated with increased susceptibility to bacterial infections and increased risk for chronic pulmonary disease. Obesity is known to increase sensitivity to endotoxin-induced liver injury, but effects on the lungs are uncharacterized. To identify the key biological pathways that define susceptibility factors for pulmonary infection during obesity, we performed parallel exposures of normal weight (NW) and diet-induced obese (DIO) C57BL/6 mice to 0.5ug/L lipopolysaccharide (LPS) by inhalation for 1hr/d for 4 days over a period of 2 weeks. Bronchoalveolar (BAL) cytology indicated a strong macrophage response with LPS exposure, which was 50% higher in DIO mice compared to NW, while neutrophil infiltration was comparable in NW and DIO mice. Likewise, levels of inflammatory cytokines in BAL fluid were increased in DIO mice, however a couple cytokines displayed some suppression of the immune response in DIO mice (IL-12, TARC) compared to NW mice. Microarray analysis revealed that DIO reprograms the lung’s transcriptional response, altering both the number of genes and the specific molecular pathways induced or suppressed by LPS exposure. Comparison with microarray data from DIO mice exposed to cigarette smoke indicates both overlapping and unique toxicity pathways, which could be used to link exposure to outcome data. In addition, we identified genes whose expression levels are significantly different between regular and DIO sham control animals, indicating an overall suppression of the immune system and induction of heat shock proteins in the lung during DIO. These results demonstrate biosignatures of systemic inflammation and oxidative stress in obese mice, which may make them more sensitive to environmental lung toxicants. Supported by U54 ES016015.

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- **Transcriptional profiling indicated increased expression of genes associated with fatty acid β-oxidation in muscle tissue with accompanying regulation of genes involved in oxidative stress. Based on lesion-associated expression changes, Ank1 (Ank1 in repeated domain 1) and Ras (Ras-associated related with diabetes) were investigated further. Ank1 and Ras protein expression in skeletal muscle tissue demonstrated a pattern similar to that of mRNA expression and correlated well with lesion occurrence. Evaluation of Ank1 and Ras as biomarkers of skeletal muscle toxicity in other biological matrices (plasma and urine) is ongoing.*

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Kidney toxicity is a common reason drugs fail in the clinic indicating a need to improve preclinical testing. New biomarkers of kidney toxicity appear to be more sensitive and region-specific than common clinical chemistry indicators such as serum urea nitrogen and creatinine. The current study was performed to compare several new urinary biomarkers of kidney toxicity to standard clinical pathology tests to determine if the new biomarkers are more sensitive for detecting kidney effects, if they provide region-specific information about injury, and if they correlate to recovery from injury. Rats were treated with one of two known nephrotoxins and urine samples from pregnant rats that received single or multiple doses of resveratrol. This study demonstrated that resveratrol treatment has an impact on endogenous metabolism.

Resveratrol, a naturally occurring polyphenol found in plants such as grapes and peanuts, has a variety of beneficial health effects. The potential for widespread exposure in diet or supplements has raised concern about possible toxicity. This study was conducted to evaluate the effects of resveratrol exposure on endogenous metabolism by examining relevant marker metabolites excreted in urine from rats administered resveratrol at doses proposed for toxicity testing in rats. A single dose of trans-resveratrol was administered p.o. (0, 78 and 1250 mg/kg) to male and female Wistar Han rats. Resveratrol was also administered daily to pregnant Wistar Han rats on gestation day (gd) 18, or from gd 11 – 18 (0, 78 and 1250 mg/kg/day). Analyses were measured by 1H NMR spectroscopy in urine that was collected in metabolism cages from 3 rats per group for up to 48 hr. Two approaches for data analysis were used: binning, and library matching. Library matching was conducted with Chenomx software, and data reduction and analysis was conducted with SAS and Umetrics software. The measured metabolites in urine could distinguish the control group from the low-dose group, and from the high-dose group for the male, the female, and the pregnant rat. The metabolic profile could also distinguish urine samples from pregnant rats that received single or multiple doses of resveratrol. This study demonstrated that resveratrol treatment has an impact on endogenous metabolism.

Exposure to drugs and/or environmental toxicants that exceed an individual's capacity or ability to metabolize and eliminate active metabolites may have a significant impact on toxicity and the dysregulation of homeostasis and the development of exposure-related human disease. Significant differences exist between individuals at the population level based upon their inherited genetic (single nucleotide polymorphisms and copy number variants) and/or epigenetic differences in environmentally responsive genic and non-genic sequences and pathways. To better understand these differences, will be begin with an overview of the current research, new strategies, and models for pharmacology and toxicology using genetically defined and/or genetically altered inbred mouse models. These genetically diverse cell or tissue based models will be used to highlight acute or chronic human disease in large genetically diverse human populations. Together, the speakers will provide both insight and new hypotheses for the role of individual (heritable SNPs, CNV, methylated sequences, sRNAi, etc.) and environmental factors that affect the development of major polygenic human diseases including asthma, drug induced liver injury, respiratory, cancer, and cardiovascular diseases.

The NTP Host Susceptibility Initiative is focused on multidisciplinary research focused on the genetic basis for individual differences in response to environmental exposure to toxic agents and the development of complex disease (e.g., asthma, cancer, cardiovascular, lupus, organ failure, obesity and diabetes, etc.). The main goal of this research is to identify causally related genes and their variant isoforms in animal models as surrogates for human exposure and to develop predictive tools for genetic based hazard identification and risk extrapolation. Based on the dense genotyping of 15 strains of inbred mice referenced to the sequenced C57BL/6j mouse, more than 8 million SNPs and copy number variants have been described and more await discovery. This presentation will focus on inbred strain and subline related differences in response to model environmental chemicals and drugs that show a 10X or greater difference in kinetic parameters for ADME, genotoxicity and haptotoxicity, and non-neoplastic and neoplastic disease phenotypes. By determining the variable range of response of quantitative measures of toxicity in multiple inbred strains of mice and performing haplotype-phenotype association analysis, we aim to identify and functionally validate the causally related mouse genes and human orthologues genes that cause or modify an individual's response to toxicity and disease. By establishing the genetic basis for the observed modes of action, we will aid across species extrapolation for hazard identification and characterization to support and improve prediction of human risk.
Panels of inbred strains of mice have been shown to encompass a genetic and phenotypic diversity that approximates that found in the human population. This variability across mouse strains extends to the response observed after treatment with pharmaceuticals, both in terms of efficacy and toxicity. Use of the antidepressant fluoxetine revealed strain-dependent differences that reflected the clinical variability associated with selective serotonin reuptake inhibitors. Likewise, the idiosyncratic nature of acetaminophen-induced liver injury has also been accurately reproduced in the inbred mouse strains. Underlying the variability of drug response and injury are significant differences in the abilities of each of the strains to metabolize and detoxify the compounds they are exposed to. A characterization of hundreds of small molecules and metabolites in the blood and liver of 35 inbred strains revealed significant strain-specific differences. Furthermore, by combining whole genome transcriptional data from 35 strains of inbred mice with a detailed haplotype map of those same strains, a strain-specific variation in the expression of an entire cluster of phase II metabolic enzymes, the glutathione S-transferase mu family of genes, was uncovered. The cataloged variation within the human glutathione S-transferase mu orthologs has been associated with increased susceptibility to drug-induced liver injury. If preclinical models are to predict outcomes in humans they must properly replicate those susceptibilities and predispositions found in the human population starting at the genetic level. This early work suggests that the inbred mouse diversity panel could provide such a resource.

The arylic hydrocarbon receptor (AhR) has been traditionally associated with regulating responses to a variety of environmental chemicals. However, the AhR has been highly conserved throughout evolution and there is a growing body of evidence that the receptor modulates critical aspects of cellular function that are independent of its response to xenobiotics. The modulation of cell responses are highly context specific resulting in growth promotion in certain cell types and growth arrest and differentiation in other cells. Endogenous chemicals have been identified in animals with AhR agonist activity indicating they are endogenous ligands for this receptor. These results suggest that the AhR should be viewed in the same light as other cellular receptors (e.g., ER, AR, and PPAR) with a physiological role that can be disrupted by xenobiotic chemicals rather than a receptor that evolved primarily as a xenobiotic sensor. Therefore, we will address new research on the biological roles for the AhR in cell growth, death, and differentiation and the potential human health risks and therapeutic benefits associated with exposure to exogenous AhR ligands. Molecular aspects of AhR signaling are conserved across other nuclear receptor pathways and therefore the issues discussed may have relevance to the modes-of-action for xenobiotics mediated by other nuclear receptors. This session will be of interest to investigators and regulators wanting to understand the latest research on the underlying biological roles for this remarkable pleiotropic receptor.

New population-level models are beginning to support a more advanced understanding of how an exposure to chemicals differs among individuals and the identity of the genetic factors that determine exposure sensitivity. Using various population-level mouse models containing genetic diversity equal or greater than in the human population, we will present data showing that genetic variation has a significant impact on the molecular and phenotypic measures, which adds an important dimension to toxicology. Specifically, we proposed and validated a strategy using a Mouse Model of the Human Population (MMHP) to identify genetic polymorphisms and novel mechanisms contributing to xenobiotic-induced liver injury using acetaminophen and ethanol as model hepatotoxins. To identify the genetic causes of acetaminophen-related liver injury, we employed whole-genome haplotype association analysis in the MMHP and discovered that polymorphisms in Ly86, Cd44, Cd59a, and Capn8 are candidate genes. We then confirmed that variation in the orthologous genes is associated with susceptibility to acetaminophen in humans. These studies support the idea that a genetically diverse MMHP can be useful as a model to understand and predict adverse toxicity in humans.
**1340 FUNCTIONAL CROSS-TALK BETWEEN AHR AND WNT SIGNALING: OPPORTUNITIES TO MODULATE EPITHELIAL AND MESENCHYMAL INTERACTIONS.**

R. L. Tangney, Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.

Proper interactions between adjacent epithelial and mesenchymal cell layers are critical for the development and maintenance of complex vertebrate structures. Recent results obtained in zebrafish regenerating tissues and in the developing mouse ventral prostate indicate that there is functional cross talk between the AHR and Wnt signaling pathways. This presentation will demonstrate the value of integrative research across models to help unravel signaling networks and will suggest that the AHR may be an entry point to therapeutically modulate Wnt signaling.

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**1341 THE ARYL HYDROCARBON RECEPTOR HAS A NOVEL ROLE IN THE MAINTENANCE AND FUNCTION OF HEMATOPOIETIC STEM CELLS AND POSSIBLY OTHER STEM CELL POPULATIONS.**

T. A. Gastwirtz, Department of Environmental Medicine, University of Rochester, Rochester, NY.

A common and defining characteristic of stem cells is the ability to supply tissues with progenitors that differentiate into mature lineages, while maintaining stem cell pools, to satisfy the demands throughout the lifetime of an organism. We have uncovered evidence to indicate that the AHR is important for regulating the balance between hematopoietic stem cell (HSC) quiescence and proliferation through the ability to modulate critical genes necessary for these cells to sense signals in the bone marrow environment. Further defining the precise role of the AHR in HSCs and other tissue stem cells will lead to the identification of previously undefined functions of this transcription factor in particular diseases, and could have important implications for their diagnosis and treatment. Given that the AHR is ligand activated also offers that opportunity to develop selective modulators that may be useful for the maintenance of stem cells and their use in tissue regeneration.

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**1342 DEVELOPMENT OF SELECTIVE AHR MODULATORS (SAHRMs) FOR TREATMENT OF DISEASE.**

S. H. Saff, Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

Ligand-activated receptors are extensively used as targets for developing tissue-selective drugs for treatment of multiple diseases including cancers. We have developed novel AHR ligands within the 6-Alkyl-1,3,8-trichlorodibenzofurans and substituted dioxin-methanes structural classes that act as selective AHR modulators (SAHRMs) and inhibit tumor cell growth and metastasis while remaining relatively nontoxic. The development of SAHRMs indicates that the AHR can be controlled like other ligand-activated receptors and opens the door for the AHR as a therapeutic target for disease treatment.

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**1343 MECHANISMS OF CHEMICAL-INDUCED LIVER CANCER: PUTTING THE PIECES TOGETHER.**

C. Corton1 and J. Goodman2, 1NH/ERL, U.S. EPA, Research Triangle Park, NC and 2Michigan State University, East Lansing, MI.

A large number of chemicals, including non-genotoxic compounds, increase the incidence of liver tumors in mice and rats. Key events in liver tumor formation include perturbation of gene expression homeostasis, increases in oxidative stress, and activation of cell growth pathways. The mechanisms that underlie these events may include activation of pathways under control of nuclear receptors. Although hepatocellular carcinoma (HCC) in humans is the fifth most common neoplasm worldwide and the third most common cause of cancer-related death, the human relevance of the rodent liver tumor response remains controversial. Differences in nuclear receptor levels and downstream responses between rodents and humans might contribute to a species difference in sensitivity. A number of new techniques that interrogate changes in the epigenome have been applied to rodent liver carcinogenesis and are illuminating the molecular events in the “black box” between nuclear receptor activation and liver tumor induction. These techniques can assess changes in the methylation status of the DNA, gene expression, alternative splicing, and miRNA levels. Information from these data streams can be integrated into mathematical models of the structure and function of the liver to identify genetic networks required for liver tumor induction and allow prediction of the ability of chemicals to induce liver cancer through different modes of action. Our panel of experts will discuss how the assessment of genetic and genomic changes have increased our understanding of the key events and human relevance of rodent liver tumors. We will conclude with a discussion of computational strategies to integrate different types of data in biologically-relevant models of hepatic functions that can be used to predict liver cancer after chemical exposure. This session will be of interest to those in systems biology, liver toxicity, nuclear receptors, and the impact of modulation of stress pathways on chemical toxicity.

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**1344 IDENTIFICATION OF GENETIC DETERMINANTS OF SUSCEPTIBILITY TO LIVER TUMOR INDUCTION.**


Genetic factors determine gender-dependent susceptibility to liver cancer. Sex hormones influence the susceptibility of inbred mice to liver cancer. C57/BR(c/d) (BR) females are extremely susceptible to spontaneous and chemically induced liver tumors, in part due to a lack of protection against hepatocarcinogenesis normally offered by ovarian hormones. BR males are also moderately susceptible, and the susceptibility of both sexes of BR mice to liver tumors induced with N,N-diethylnitrosamine relative to the resistant C57/BL(6) (B6) strain is caused by two loci designated Hcfl and Hcfl2 (hepatocarcinogenesis in females) located on chromosomes 17 and 1, respectively. The Hcfl locus on chromosome 17 is the predominant modifier of liver cancer in BR mice. To validate the existence of this locus and investigate its potential interaction with Hcfl2, congenic mice for each region were generated. Homozygosity for the B6.DR(B17/Mat164-D17Mit2) region resulted in a 4-5-fold increase in liver tumor multiplicity in females and a 4.5-fold increase in males compared with B6 controls. A series of 16 recombinants covering the entire congenic region was developed to further narrow the area containing Hcfl. Susceptible heterozygous recombinants demonstrated a 3- to 7-fold effect in females and a 1.5- to 2-fold effect in males compared with B6 siblings. The effect in susceptible lines completely recapitulated the susceptibility of heterozygous full-length chromosome 17 congenics and furthermore narrowed the location of the Hcfl locus to a single region of the chromosome from 30.05 to 35.83 Mb. The potential role of candidate genes within this region in affecting liver cancer will be discussed.

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**1345 IDENTIFICATION OF GENES INVOLVED IN PHENOBARBITAL-INDUCED CARCINOGENESIS: EMPHASIS ON ALtered DNA METHYLATION, EXPRESSION, AND PATHWAYS.**

J. J. Goodman. Pharmacology & Toxicology, Michigan State University East Lansing, MI.

Aberrant DNA methylation, an epigenetic mechanism, facilitates carcinogenesis. Hypermethylation can silence tumor suppressors, while hypomethylation might up-regulate oncogenes. We utilize 2 model systems to investigate the effects of the classical nongenotoxic rodent liver carcinogen phenobarbital (PB) on methylation patterns and gene expression in susceptible mice, as compared to their resistant counterparts. We hypothesize that at least some of the changes that occur uniquely in the sensitive mice play critical mechanistic roles underlying tumor formation. While the fundamental genes underlying carcinogenesis in mice and humans are likely the same, rodents exhibit an increased susceptibility, and methylation patterns in rodent cells seem less stable than in human cells. Thus, a difference between humans and rodents might exist with regard to the regulation of epigenetic control, including DNA methylation, resulting in enhanced sensitivity to tumor formation in the latter. Treatment with PB (0.05% in water) resulted in unique regions of altered methylation (RAMs) in susceptible B6C3F1 (as compared to relatives resistant C57/BL(6) mice at 2 and 4 wk, and in susceptible constitutive acetoacetate receptor (CAR) wild-type (WT), as compared to resistant knockout (KO) mice, in both precancerous liver tissue and individual liver tumors at 4, 23 and 32 wk, respectively. Unique gene expression changes were discerned in the tumor-prone B6C3F1 and CAR WT, as compared to the resistant groups, e.g., perturbations of DNA methyltransferases, indicating a mechanistic link between PB and altered methylation, and genes within the cell cycle, plus key signaling pathways, including mitogen-activated protein kinase (MAPK), TGF-beta and Wnt. Using 2 model systems, we have examined alterations in DNA methylation and expression of key genes that occur across a continuum of PB-induced liver tumorigenesis. Our data suggest that PB affects both DNA methylation and critical signaling pathways uniquely in liver tumor-susceptible mice, potentially driving carcinogenesis.
**1346** THE OTHER WORLD OF THE TRANSCRIPTOME: ROLE OF NUCLEAR RECEPTORS IN CHEMICAL-INDUCED EFFECTS ON ALTERNATIVE SPlicing IN THE LIVER.

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Alternative splicing (AS) is a form of epigenetic regulation that enables a single gene to give rise to multiple, differentially spliced versions from a single primary transcript. A large number of genes important in xenobiotic metabolism and cancer are regulated by members of the nuclear receptor family (constitutive activated receptor (CAR) and peroxisome proliferator-activated receptor alpha (PPARalpha)) as well as oxidant-activated Nrf2. Very little is known about the relationships between activation of these transcription factors, AS of target genes and toxicological and pharmacological effects of these chemical activators. We have used full-genome exon arrays to assess AS in the livers of wild-type mice and mice nullizygous for CAR, PPARalpha and Nrf2 after chemical exposure. Our results indicate that 1) large numbers of genes exhibit AS but not necessarily changes in gene expression after chemical exposure, 2) there are overlaps in the genes exhibiting AS by different xenobiotics, 3) many of the AS events would be predicted to alter protein structure, 4) there is a bias in the AS events at the 5' and 3' ends of the regulated transcripts, 5) there are exonic “hot spots” for AS that are chemical independent and 6) the changes in AS were receptor-dependent. These results demonstrate that xenobiotic exposure leads to extensive changes in AS in genes involved in xenobiotic metabolism and cell growth. An analysis of the AS events after chemical exposure may be useful for predicting the changes in the expression and structure of the proteome which plays a more proximal role in chemical carcinogenesis. (This abstract does not necessarily reflect U.S. EPA policy).

**1347** IMPACT OF ALTERED MICRORNA EXPRESSION IN LIVER CARCINOGENESIS.

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Environmental exposure to natural and man-made chemicals is one of the major causes of human cancer. It is widely believed that genotoxic and non-genotoxic alterations induced by some of these carcinogens are critical for tumorigenesis. Currently, in addition to the genetic and epigenetic mechanisms in the transmission of genetic information, extensive studies have indicated the existence and importance of another mechanism in regulation of gene function mediated by microRNAs (miRNAs). We have conducted experiments to examine the role and contribution of miRNA alterations to rodent liver carcinogenesis induced by genotoxic and non-genotoxic carcinogens. Long-term exposure of rats to the genotoxic carcinogens, tamoxifen and 2-acetylaminofluorene, and to a non-genotoxic carcinogenic methyl-deficient diet resulted in the substantial alterations of the miRNA expression profiles. The targets for these miRNAs are known to affect cell proliferation, apoptosis, lipid metabolism, oxidative stress, DNA methylation, and inflammation. Among differentially expressed hepatic miRNAs induced by exposure to genotoxic and non-genotoxic carcinogens, miR-34a, miR-155, miR-200b, miR-200c, and members of the miR-17-92 oncogenic cluster were the most up-regulated. Interestingly, each of these miRNAs is involved in the regulation of apoptosis or cell proliferation, most frequently disturbed pathways during liver carcinogenesis. In this context, the altered expression of miR-34a, miR-155, miR-200b, miR-200c, and miR-17-92 cluster illustrates the critical role of miRNAs in the disruption of the delicate balance between cell proliferation and apoptosis during carcinogenesis. These results demonstrate that alterations in expression of miRNAs are a prominent fundamental event during early stages of liver carcinogenesis induced by genotoxic and non-genotoxic carcinogens. More importantly, our data mechanistically link alterations in miRNA expression to the pathogenesis of liver carcinogenesis.

**1348** PREDICTIVE MODELS OF LIVER CANCER.


Predictive models of chemical-induced liver cancer face the challenge of bridging causative molecular mechanisms to adverse clinical outcomes. The latent sequence of intervening events from chemical insult to toxicity are poorly understood because they span multiple levels of biological organization and timescales. The availability of high-throughput molecular assays provide a global view of epigenetic, transcriptional and pathological level changes that can shed much needed light on the regulatory networks perturbed by xenobiotic stressors. A key challenge in this process is to resolve the role of these networks in the normal homeostatic response of cells as opposed to irreversible alterations due to persistent stress. To link molecular mechanisms to neoplastic lesions will require modeling the altered cellular phenotypes, as that is the level of biological organization at which tissue damage becomes manifest. This talk will outline our implementation of this multi-scale approach in the U.S. EPA Virtual Liver (v-Liver(TM)) – a cellular systems model of hepatic tissues aimed at predicting chemical-induced histopathologic effects through agent based simulations. The v-Liver(TM) is part of a broader community effort to develop in silico models of the cellular fabric of living tissues, called Virtual Tissues (VT3). As a proof of concept we are using molecular and cellular data on 20 nuclear receptor (NR) activating hepatocarcinogens from the EPA ToxCast(TM) Program and short-term in vivo studies. The first part of this talk will discuss the integration of mechanistic knowledge with high-throughput screening and -omic data to model the effects of NR-mediated networks on hepatocyte injury, death and proliferation. The second part of the talk will present the integration of pharmacokinetic (PK) and cell-level data to simulate dose-dependent hepatic effects. This work was reviewed by EPA and approved for publication but does not necessarily reflect official agency policy.

**1349** INTRODUCTION TO THE HESI IVGT PROJECT COMMITTEE.

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The field of genetic toxicology is in need of new approaches in experimental design and data interpretation to improve the scientific basis of its utility for the purpose of accurate human risk assessment. Furthermore, there is an urgent need for a framework for the integration of the in vitro testing results into a risk-based assessment of the effects of chemical exposures to human health. A tripartite initiative under the auspices of the ILSI Health and Environmental Institute involving scientists from regulatory, academic, and industrial sectors was initiated to address and make recommendations on these issues. The scientists involved in this initiative were charged with systematically examining the state of the science in genotoxicity assessment, assessing the utility of new and emerging genetic toxicology tools, and addressing a shift away from qualitative genotoxic assessment to a quantitative approach. The recommendations emerging from this initiative as well as those advanced by others are expected to advance the field of genetic toxicology into the 21st century.

**1350** CURRENT STRATEGIES IN ASSESSING GENOTOXIC RISK.

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The current battery of genotoxicity tests for assessing the potential of chemicals to induce genetic damage/alterations has been in use since the mid-1980s and early 1990s and serves regulatory purposes well for identifying and screening for genotoxicity. Currently, there are two primary tests: the chromosome aberration test and the unscheduled DNA synthesis test. These newer insights will enhance and improve the current testing schemes and strategies.

**1351** NEED FOR A NEW APPROACH TO GENETIC TOXICITY ASSESSMENT: LESSONS LEARNED AND NEW OPPORTUNITIES.

J. T. MacGregor, Toxicology Consulting Services, Arnold, MD.

The need for quantitative dose-response information to inform risk assessment was recognized when regulatory genetic toxicology testing guidelines were introduced in the 1970s, but the lack of routine in vivo test methods and over-enthusiasm about the ability of in vitro methods to identify carcinogens led to regulatory guidelines based on qualitative characterization of genotoxic potential. Current in vitro test methods qualitatively identify potent DNA-damaging agents, but do not allow reliable extrapolation to human risk as a function of exposure. Advances in methodologies for monitoring in vivo mutations and chromosomal damage offer the opportunity for an improved and cost effective paradigm of genotoxic risk assessment. A quantitative exposure-based risk assessment requires definition of appropriate exposure and response metrics, and metrics to normalize exposures and responses across different test systems in vivo and in vitro. Case examples, including responses to small molecular weight alkylating agents and polycyclic mutagens, suggest that
of exposure metrics such as Cmax, AUC, or DNA or protein adducts may be useful to normalize exposures across test systems, and that normalizing mutagenic response to cellular toxicity (by assuming a constant ratio of toxic to genotoxic effects across systems) may also facilitate extrapolation across in vitro and in vivo test systems. The ILSI/HESI initiative is assembling a data base to evaluate exposure-response relationships in different assays, and to define appropriate metrics for quantitative approaches. Initial data suggest that some DNA-reactive mutagens have practical no-effect levels and that normalization across test systems may be feasible in certain carcinogenicity and/or carcinogenicity studies. The relevance of such in vitro findings for humans has been questioned. A tripartite initiative under the auspices of the ILSI Health and Environmental Sciences Institute involving genetic toxicology experts from regulatory, academic, and industrial sectors addressed the above issues and came up with a recommended approach to address them. Some aspects of the above issues, especially those dealing with the in vivo studies, were further deliberated at the International Workshop on Genotoxicity Testing held in August of 2009. Existing assays have been reviewed to re-examine the strengths, and weaknesses, and classify the assays according to their ability to contribute to genotoxicity risk assessment. General conceptual flow-charts have been constructed to define follow-up actions to pursue when clear positive results are obtained in genotoxicity assays. These frameworks take into consideration a review of all existing data and information, including mode of action, intended usage, and human exposure. They then elaborate on the potential utility of additional information that needs to be generated, both in vitro and in vivo, to help answer specific questions and better determine the risk for human. These strategies are expected to provide a weight of evidence framework to determine the relevance of the positive findings from genotoxicity assays to humans.

New Technologies to Predict Genotoxic Risk in Humans.

D. Jacobson-Kram, U.S. FDA, Silver Spring, MD.

Components of routinely used genotoxicity test batteries have been largely unchanged for a quarter century. While the traditional 3-test battery has been shown to have very high sensitivity for predicting rodent carcinogenicity, concerns over low specificity have been raised. Low specificity can result in discarding potentially useful new molecules or triggering additional testing generally performed in intact animals. The advent of new “omic technologies (e.g. expression arrays) and new assays promises to deliver more accurate, more relevant and mechanistically based information on human risk.

Beyond Positive or Negative: A Quantitative Approach for Interpreting Genotoxicity Data.

B. Gollapudi, Dow Chemical Company, Midland, MI.

Genotoxicity data are usually interpreted in a binary mode, i.e., whether a chemical is positive or negative for inducing a response in the test system. Although dose-response information is sometimes used in arriving at the binary decision, it has not been a routine practice to use such data in deriving any quantitative information to or to establish no-observed-genotoxic-effect levels (NOGELs). This is primarily due to the hitherto mindset of considering most genotoxic responses as linear data. Currently used methods for assessing genotoxicity are designed in order to obtain precise information at the lower end of the dose-response curve. There is now a growing body of evidence to support the existence of NOGELs/thresholds for a number of materials, including such DNA-reactive agents as MMS, EMS, MNU, and ENU, both in vitro and in vivo. With the type of technology available today, it is now possible to obtain accurate information on the dose-metric to enable valid comparisons of NOGELs across test systems. It is recognized that simple demonstration of a “hockey-stick” shaped dose-response curve per se is not sufficient to establish thresholds for a chemical and it is necessary to establish the biological plausibility for the observed threshold effect. Chemical-specific mode-of-action information that identifies key events responsible for the observed effect will facilitate comparison of data across the test systems as well as enabling to assess the relevance of the observed effect to humans. Biomarkers of key events that provide qualitative as well as quantitative information can be integrated at various decision points in a weight-of-evidence based assessment of genotoxicity derived from multiple test systems and to identify any follow-up studies to resolve/reduce uncertainties during the risk assessment process.

Optimal Design for In Vivo Mutation Studies to Inform Cancer Mode-of-Action Assessment.

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Mutation data obtained in vivo is particularly relevant for providing information to inform the mode-of-action (MOA) assessment for chemicals that are both mutagens and carcinogens. The outcome of the MOA determination can impact the model used for the quantitative risk assessment and therefore, it is particularly important that MOAs be properly determined. There is a large database for chemicals that have been evaluated in vivo in mutation models. Unfortunately when one looks closely at the data that has been generated to date, it becomes clear that only rarely is there data from the cancer target tissue and from doses that are similar to those used in the cancer bioassay. An overwhelming number of these studies were conducted using a design (particularly in terms of dose selection) that is optimized for hazard identification and often is not ideal for assessing MOA. In particular, there is a focus on high doses that are often above those required to see tumors in the cancer bioassay. It is critical to in vivo mutation study designs be optimized to inform MOA assessment rather than just hazard identification. The presentation will outline the differences between the current study designs for hazard identification and designs more optimal for MOA assessment.

Recent Knowledge on Critical Regulators of Lipid Homeostasis in Metabolic Disease.

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Modifications in human lifestyle and nutritional status over the recent decades have lead to an increase in the incidence of obesity and insulin resistance, leading to metabolic syndrome. Metabolic syndrome is a co-morbid condition of risk factors for diseases such as diabetes, hepatic steatosis, cardiovascular disorders, stroke, and drug-induced toxicities. Insulin resistance, a hallmark of metabolic syndrome, is thought to play an important role in the development of hyperglycemia, hyperlipidemia, and lipotoxicity. Metabolic abnormalities such as insulin resistance, lipotoxicity, and hyperglycemia are not only associated with lifestyle changes but also by consumption of xenobiotics such as ethanol. The accumulation of highly toxic lipid metabolites has been shown to contribute towards ER stress and organ toxicities. In the past, various strategies for increasing tissue glucose uptake and metabolism and fatty acid oxidation have been devised for normalizing the elevated blood glucose and lipid levels thereby improving insulin sensitivity and the metabolic syndrome. Metabolic syndrome is a culmination of risk factors for diseases such as diabetes, obesity, stroke, cancer, and it is necessary to establish the biological plausibility for the observed threshold effect. Chemical-specific mode-of-action information that identifies key events responsible for the observed effect will facilitate comparison of data across the test systems as well as enabling to assess the relevance of the observed effect to humans. Biomarkers of key events that provide qualitative as well as quantitative information can be integrated at various decision points in a weight-of-evidence based assessment of genotoxicity derived from multiple test systems and to identify any follow-up studies to resolve/reduce uncertainties during the risk assessment process.
Alcoholic fatty liver represents a critical, initial stage in the progression of alcoholic liver disease. This early, generally benign condition can eventually lead to development of more serious conditions such as hepatic fibrosis and cirrhosis. Although significant advances have been made in understanding the molecular basis of alcoholic fatty liver, the precise mechanisms are still not completely understood. Sirtuin 1 (SIRT1), a class III NAD+-dependent protein deacetylase, is emerging as a master lipid regulator. The fact that SIRT1 requires elevated ratios of NAD+/NADH for its enzymatic activity implies a potential link between ethanol metabolism and SIRT1 in the liver. Ethanol-mediated inhibition of hepatic SIRT1 leads to impairment of hepatic fat metabolism through modulation of several transcriptional regulators including AMP-activated kinase (AMPK), sterol regulatory element binding protein 1 (SREBP-1), peroxisome proliferator-activated receptor alpha (PPAR-α) and PPAR-gamma co-activator-alpha (PGC-1α), thereby contributing to increased hepatic lipid synthesis, reduced fatty acid oxidation and development of fatty liver. These studies suggest that stimulation of SIRT1 could be an effective mechanism for treating alcoholic fatty liver, and that synthetic, therapeutic agents (perhaps similar to dietary polyphenols such as resveratrol) could be developed to this end.

PPAR binding protein (PPB/MED) is a transcriptional co-activator for several nuclear hormone receptors, including the Peroxisome Proliferator-Activated Receptor family (PPARs) and thyroid hormone receptors (TRs). PPB has been previously identified as a component of the TRAP/DRIP/Mediator complex that participates in transcriptional activation of target genes, in part, via interaction both with the RNA polymerase II complex and transcription factors. In vivo deletion of PPB in the heart leads to the rapid development of cardiomyopathy secondary to disruption of processes critical for maintenance of normal mitochondrial function and number. The mechanism by which this is accomplished involves both nuclear hormone receptor- and non-nuclear hormone receptor-mediated transcriptional programs that regulate fatty acid handling and metabolism, TCA cycle components, and enzymes of oxidative phosphorylation. PPB is revealed as a critical component of the signaling pathways that control cardiac mitochondrial biogenesis and represents an integrator of hormone and energetics-driven response to the metabolically plastic cellular environment of the cardiac myocyte.

Lipins are identified by using a positional cloning approach to localize the causative mutation in the fatty liver dystrophic (fld) mouse. mice genetically-deficient in lipin 1 (fld mice) exhibit life-long deficiencies in adipose tissue development, neonatal hepatic steatosis, insulin resistance, and increased susceptibility to developing atherosclerotic lesions. Like AMPK and TOR, PARS kinase (PASK) is also a nutrient-responsive protein kinase. In yeast, PARS kinase phosphorylates the enzyme Ugp1 and thereby shifts glucose partitioning toward cell wall glucan synthesis at the expense of glycogen synthesis. Consistent with this function, yeast PARS kinase is activated by both cell integrity stress and growth in non-fermentative carbon sources. PARS is also important for proper regulation of glucose metabolism in mammals at both the hormonal and cellular level. PAS kinase plays a central role in mediating some of the nutrient effects on fatty acid synthesis and metabolism. Also, it plays a novel role in non-alcoholic fatty liver disease.

Metabolic syndromes are featured by a group of metabolic risk factors in one person, including abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance, and proinflammatory state. While the exact mechanism remains elusive, an altered homeostasis of essential metals such as zinc (Zn) and copper (Cu) is known to contribute to the etiology of metabolic syndromes. Zn and Cu are the metals indispensable for the structure and activity of many enzymes and proteins; their deficiency and overload have been associated to numerous patho-physiological changes, including insulin resistance syndrome. Zn deficiency is closely related to the metabolic syndrome, cardiovascular diseases, and insulin resistance, while increased systemic Cu levels may be related to the risk of cardiovascular disease, brain diseases, and other metabolic syndromes. The current understanding of the roles of Zn and Cu homeostasis in the insulin signaling, cardiovascular inflammation, diabetes, and diabetic complications will be explored. A brief overview highlighting the association of Zn and Cu with inflammation, diabetes, and diabetic complications will begin this session. Important components of this exploration will cover how Zn sensitizes insulin function and protects endothelial cells from oxidative stress. The effect of Zn dyshomeostasis on cardiac mitochondrial dysfunction, oxidative stress, and pathogenic remodeling will then be examined. The dysregulation of Cu homeostasis in brain and cerebrospinal fluid as the consequence of iron (Fe) metabolic disorders will also be discussed. Given that metallothionein (MT) plays a critical role in Zn homeostasis, therefore, how Zn via MT’s gene upregulation was used to prevent diabetes and diabetic complications will be addressed in the final presentation.
endothelial function. The role of zinc deficiency in atherosclerosis is not well defined. Zinc has antioxidant and anti-inflammatory properties in vascular tissues, and our data suggest that zinc is crucial for the protection against cell-destabilizing agents, such as polyunsaturated fatty acids and inflammatory cytokines. For example, oxidative stress was increased markedly in zinc-deficient endothelial cells following treatment with linoleic acid or TNF-α, and this increase in oxidative stress was partially blocked by prior zinc supplementation. In addition, activated endothelial cells demonstrated increased DNA binding activity of NF-κB and AP-1, which was lowered considerably when cells were supplemented with physiological levels of zinc. Similarly, endothelial cell production of IL-6 was increased in zinc-deficient endothelial cells, which was partially blocked by zinc supplementation. Most importantly, zinc can modulate peroxisome proliferator activated receptor (PPAR) function. We found that zinc deficiency decreased PPAR activation and protein expression. In contrast, zinc supplementation markedly increased PPAR activation and expression, which was correlated with down-regulation of DNA binding activity of NF-κB and AP-1. These studies were confirmed in an atherosclerotic mouse model, where PPARα signaling was compromised during zinc deficiency. For example, ROS/ATP1-induced induced inflammatory genes (e.g., MCP-1) only during zinc deficiency, and adequate zinc was required for ROS/ATP1 down-regulate pro-inflammatory markers. Our data suggest that there exists a complex interaction among pro-and anti-inflammatory transcription factors and associated cellular signaling, which requires adequate zinc for regulatory functions towards an anti-inflammatory outcome of these metabolic events. (Supported by NIEHS, NIH (P42ES07300) and the University of Kentucky AES).

1364 COUPLED CALCIUM AND ZINC DYSHOMEOSTATE IN CARDIAC MYOCYTES AND MITOCHONDRIA DURING CHRONIC ALDOSTERONISM.

K. T. Weber. Division of Cardiovascular Diseases, University of Tennessee Health Science Center, Memphis, TN. Sponsor: L. Cai.

Congestive heart failure (CHF) is a syndrome whose symptoms and signs are rooted in an inappropriate (relative to dietary Na+) activation of the circulating renin-angiotensin-aldosterone system that ensues when the failing heart does not sustain adequate renal perfusion. This homeostatic mechanism gone awry begets Na+ and water retention, a dyshomeostasis of macro- and micronutrients, and cardiac pathology. To better understand the pathophysiologic consequences of chronic aldosteronism, rats receive aldosterone from an implanted minipump to raise its blood levels to those found in human CHF together with 1% NaCl in drinking water fortified with 0.4% KCl to prevent hypokalemia. This model is referred to as aldosterone/salt treatment (ALDOST). A dyshomeostasis of extra- and intracellular Ca2+ accompanies ALDOST. Secondary hyperparathyroidism with parathyroid hormone-mediated intracellular Ca2+ overload occurs in diverse tissues in response to ionized hypokalemia precipitated by increased urinary and fecal Ca2+ excretion. In the case of cardiomyocytes and their mitochondria, we hypothesized that this excessive intracellular Ca2+ accumulation is intrinsically coupled to zinc translocation where Ca2+ serves as prooxidant and Zn2+ as antioxidant. In response to chronic (4 weeks) ALDOST and compared to untreated, age-sex-matched controls, we found: a) increased cardiomyocyte and mitochondrial Ca2+ and Zn2+, together with biomarker evidence of oxidative stress and increased activity of antioxidant defenses; b) concomitant upregulated expressions of metallothionein-1, Zn2+ transporters (Zip1 and ZnT-1), and metal-responsive transcription factor-1 and c) myocardiocellular necrosis, a feature of earlier cardiomyocyte necrosis. These findings call into question potential cardioprotective strategies (e.g., a Zn2+ and/or Ca2+ supplements or Ca2+ channel blockers) that could uncouple these two divalent cations during aldosteronism and thereby modulate their ratio in favor of sustained antioxidant defenses to prevent cardiomyocyte necrosis and scarring.

1365 REGULATION OF COPPER HOMEOSTASIS IN BRAIN AND CEREBROSPINAL FLUID: EFFECT OF IRON DEFICIENCY AND OVERLOAD.

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A stable Cu homeostasis is critical to brain’s normal function. Systemic Fe deficiency (Fe-D) or overload (Fe-O) either as the cause or consequence of metabolic/environmental diseases can have a profound effect on brain Cu level. Two types of brain barrier systems control the Cu uptake into and removal from brain inner milieu; the blood-brain barrier (BBB) regulates Cu fluxes between blood and brain interstitial fluid and the blood-CSF barrier (BCB) regulates Cu transport between blood and CSF. Evidence from this laboratory showed that free 64Cu uptake in the BBB was about 50 and 1,000 times higher than 64Cu-albumin and 64Cu-ceruloplasmin uptake, respectively. The unidirectional transport constant (Kin) for Cu was much greater in the BCB or BBB than that in the CSF or capillary-depleted brain parenchyma. Hence the main entrance for Cu into the brain appears by the BBB as a free, unbound species, whereas the BBB serves the clearance pathway for excess Cu in the CSF. In rat models with Fe-D or Fe-O, a significant increase (+54%) and decrease (-58%) in CSF Cu were observed in Fe-deficient and Fe-overloaded animals, respectively. While the Fe-O treatment did not seem to affect Cu levels in brain parenchyma and choroid plexus to any significant extent, the Fe-D animals showed a significant increase in Cu levels in both brain and plexus tissues. Hence, an Fe deficiency disease status appears to have a more vigorous effect on brain Cu homeostasis. This presentation further discusses the role of the BBB and BCB in regulating Cu transport through the critical transporters, i.e., Cu transport protein-1 (Ctrl), divalent metal transporter-1 (DMT1), ATP7A and Atox1, and how the Fe-D or Fe-O disorder may affect expressions and functions of these transporters. Modulation of Cu transporters may provide the useful tool for treatment of distorted Cu homeostasis in the central nervous system in metabolic diseases. (NIEHS ES08146 and ES 017055).

1366 MODULATION OF THE METABOLIC EFFECTS OF GSK-3 ISOFORMS BY ZN AND/OR CU: IMPLICATIONS FOR DIABETES.


Intensive study over the past thirty years has helped define the role of the glycogen synthase kinase-3 (GSK-3) family in a variety of physiological and pathophysiological processes. Herein I will examine recent data derived from studies in gene-targeted mice in an attempt to define the role these protein kinases play in metabolism. For example, the GSK-3β KO demonstrates reduced fat mass with enhanced glucose tolerance and insulin sensitivity due, at least in part, to enhanced glycogen storage in the liver. While mice totally lacking GSK-3β die late in development, tissue-specific knockout of GSK-3β have revealed metabolic phenotypes in muscle including enhanced insulin-stimulated glycogen synthesis activation and glycogen deposition. Moreover, inactivation of GSK-3β in just the β-cells of the pancreatic islets is sufficient to rescue insulin resistance and largely prevent the manifestations of diabetes in various models of the disease in mice. The pancreatic β-cell knockouts also demonstrated preservation of β-cells due to enhanced proliferation and reduced apoptosis of these critical insulin-secreting cells. More recently, increasing evidence suggests that GSK-3β activity may be modulated by metallothionein, and this may play a central role in diabetic cardiomyopathy. All of these areas will be discussed in an attempt to understand more clearly some of the metabolic effects of Cu and Zn.

1367 POTENTIAL EFFECTS OF ZINC ON DIABETIC COMPLICATIONS: ROLE OF METALLOTHIONEIN.

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There is increasing evidence suggesting that oxidative stress plays the major role in the development of diabetic cardiovascular complications. Metallothionein (MT) is cysteine-rich metal-binding proteins with several suggested biological roles including antioxidant property. Both in vitro and in vivo studies have indicated that MT can act as an antioxidant in the heart and kidney to prevent any damage caused by a variety of oxidative stresses including diabetes. Studies, including the author’s own group, have demonstrated how systematically demonstrated the significant protection by MT against diabetes-induced cardiac and renal damage. Since MT is ubiquitously expressed in mammalian tissues and is highly inducible by variety of reagents such as zinc, the potential clinical application for induction of MT as an antioxidant by zinc supplementation to prevent various diabetic complications including cardiomyopathy and nephropathy has been explored in diabetic animal models. In this presentation, therefore, the protective effect of MT against diabetes-induced cardiomyopathy and nephropathy will be first introduced, based on the results from cardiac- or renal-specific MT-overexpressing mouse models. Then, the finding that cardiac and renal induction of MT synthesis by zinc supplementation in diabetic mice and rats prevented the development of diabetic cardiomyopathy and nephropathy will also be presented. The potential clinical application of MT or zinc-induced MT will be discussed in combination with the available clinical data that indicated beneficial effects by zinc supplementation against various diabetic complications.
Prophylactic vaccination has proven highly effective against many highly virulent infectious diseases and has reduced the medical burden from these infections throughout the world. Despite these successes, many infectious agents, such as human immunodeficiency virus (HIV), herpes simplex virus (HSV), and Epstein Barr Virus (EBV) establish chronic latent infections and create considerable morbidity upon reactivation following immunosuppression. Traditional vaccines that generate antibody-mediated immunity have limited effects on chronic quiescent infections and do little to inhibit the spread of these viruses. While monoclonal antibody therapy can provide limited passive vaccination for these maladies, the cost is great and patient compliance is low. A therapeutic vaccination that induces both humoral (antibody-mediated) and cellular (T cell-mediated) immunity holds promise in combating these latent infections, as well reducing the medical impact for other chronic human maladies, including cancer, addiction, and genetic/metabolic disease. In addition to cost and compliance issues, successful therapeutic vaccine will need to overcome immune tolerance while controlling dysregulation and/or deleterious effects of immune activation (i.e., unwanted T cell activation and undesirable off-target effects will need to be minimized). An overview of clinical indications under consideration for therapeutic vaccination (HIV, caffeine and nicotine addiction, cancer, etc.), approaches to development of therapeutic vaccines (adjuvant use, dendritic cell activation, viral vectors, etc.), and safety concerns of therapeutic immune activation (induction of autoimmunity, unregulated T cell activation, etc.) will be presented.

Therapeutic vaccination requires an acceptable balance between an immune response that controls/destroys a target cell or molecule and a pathological immune response that results in detrimental tissue damage. Safety considerations include evaluation of reduced antigen level effects, analysis of antibody-dependent-cell-mediated cytotoxicity, and reversibility of the induced immune response and/or unwanted T cell responses. Some development hurdles for therapeutic vaccine are safety and efficacy; safety concerns cause the most difficulty in achieving efficacy.

Immunological tolerance is a state of antigen-specific immune responsiveness (e.g. to self or innocuous environmental antigens) that does not result in tissue pathology. Many different mechanisms mediate this tolerant state (sometimes misleadingly termed “non-responsiveness”), which is essential for maintenance of homeostasis but can also result in ineffective defense against pathogens. The aim of therapeutic vaccines is to break this tolerant state and enhance beneficial immune responses to treat disease. Lack of B cell responses to self antigens is primarily ensured by T helper cell (Th) tolerance to the antigens. Strong antibody responses to self antigens can be induced by bypassing Th tolerance (e.g. by linking the B-cell epitopes to foreign carrier proteins), which can be exploited for the development of therapeutic antibody response vaccines that could serve as an alternative to monomodal antibody therapy. T-cell tolerance to self antigens is generally more complex and more difficult to overcome, but doing so may be essential for therapeutic vaccine approaches to cancer and chronic infections. Potent adjuvants and immunomodulators (e.g. antibodies, recombinant proteins, small molecules) will be needed to enhance T cell activation by antigen while controlling regulatory T-cell populations and maintaining a tolerant state to other antigens. Immune tolerance protects the body from undesirable tissue damage; thus, therapeutic vaccine approaches have a risk of increased pathology. By exploiting our growing knowledge of the molecular and cellular mechanisms underpinning the immune response in a given therapeutic setting, vaccination approaches can be designed to provide efficacy while mitigating undesirable immune effects.

This presentation will provide an overview of the current CBER perspective regarding the preclinical evaluation of therapeutic vaccine/adjuvant combinations and discuss potential regulatory and scientific challenges to assessing the safety and activity of these novel therapies.

GSK Biologics has been active in the development of Antigen Specific Cancer Immunotherapeutics (ASCIs) for the last two decades, and currently has several ASCIs in various stages of clinical development. Although therapeutic vaccines for non-infectious diseases are excluded from the scope of the WHO Guidelines on Nonclinical Evaluation of Vaccines and the EMEA Note for Guidance on Nonclinical Pharmacological Testing of Vaccines, the EMEA Guideline on Adjuvants in Vaccines for Human Use is applicable to the non-clinical aspects of “therapeutic vaccines”, including “tumour vaccines”. As no guidance yet exists that is directly applicable to non-infectious disease therapeutic vaccines, one could consider that the general principles of nonclincial toxicological evaluation outlined in each of these guidances may be considered when designing non-clinical studies to support the development of non-infectious disease therapeutic vaccines, including ASCIs. This presentation will review the design of GSK Biologics local tolerance, repeated dose, safety pharmacology and reproductive/developmental toxicology studies conducted to support ASCIs in clinical development, and will also include a discussion of species selection issues for this class of products.

Beta-amyloid (Aβ) is a fragment of amyloid precursor protein (APP), various forms of which make up the dense sticky plaque deposits in the brain that are characteristic of Alzheimer’s disease (AD). Nonclinical studies in transgenic (Tg) mouse models of AD showed that active immunization against Aβ or passive administration of anti-Aβ antibodies can result in reduction of Aβ plaque and/or amelioration of neurobehavioral effects in these animals. Active immunization of AD patients with Aβ1-42 (AN1792) slowed the clinical progression of AD; however, approximately 6% of the patients developed evidence of meningoencephalitis. A series of nonclinical and clinical investigations demonstrated that the meningoencephalitis observed in these patients was the result of the generation of Aβ-specific Th1 cells. Nonclinical models were developed that allowed for the evaluation of the relative ability of various immune responses to elicit Th1 and/or inflammatory changes in the brain of Aβ plaque-bearing mice and were used in the nonclinical safety evaluation of second generation vaccines designed to generate Aβ specific antibodies in the absence of Aβ specific T-cells.
The current discovery screening paradigm for the selection of novel molecular candidates to progress into development is fraught with a high level of failure in the early-to-late stages of development. The relationship of the number of candidates entering Phase I to achieve one successful registration varies from one pharmaceutical company to another. However, published data suggests that for every 12 new molecular entities entering Phase I, only one successfully achieves marketing authorization. The significant cost of such an endeavor in terms of operating expenses, lost time, and missed opportunities to advance the best candidates for the treatment of devastating diseases is too great to justify continuing with the status quo. As a result, there has been significant effort devoted across the pharmaceutical industry to early identification of potential liabilities of promising lead candidates that may lead to failure of those candidates in development. A goal of this session will be to advance the topic of discovery risk mitigation introduced in recent years in an attempt to reduce the number of failures being witnessed in early-to-late development. Topics include, identifying the potential on-target-related toxicities during lead finding, deselecting those candidates likely to fail in development due to on-target or target related toxicities, staged approaches to evaluating the pharmacodynamic safety (safety pharmacology) of potential lead candidates, integrating safety endpoints into proof of concept studies, and application of structure activity toxicology (SAT) identifying and mitigating the risk of metabolite-related toxicity. Presenters will share their experiences in each of these emerging areas of safety science and engage the audience in a debate of best practices. Important deliverables will include advancing knowledge in the conceptual and practical approaches to mitigating the risk of failure of promising new drugs progressing towards marketing authorization.

W 1375 MITIGATION STRATEGIES DURING EARLY RESEARCH: EVALUATION OF NOVEL THERAPEUTIC TARGETS FOR POTENTIAL ON-TARGET TOXICITY.

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Drug development is a long, complex and expensive process. Typical development timelines are between 10 and 15 years with attrition rates that are often too high for companies to sustain productive pipelines. Investigational and discovery toxicology are extensions of the field of general toxicology, created to fulfill the growing need for generating higher throughput, integrative, and predictive toxicological information, in an effort to reduce attrition at later stages of drug development. These novel ideas have been introduced as a tool to target related toxicities and have been widely adopted. The incorporation of toxicological strategies will pave the way for future drug development paradigms. This presentation will focus on the challenges and strategies applied to the evaluation of novel therapeutic targets associated with potential known and unknown toxicities based on publications, outcomes of general safety evaluations, or failure of a candidate progressing to lead optimization and development. Specific examples of target de-risking activities will be presented as will the integration of this information into the overall safety strategy of a project.

W 1376 MITIGATION STRATEGIES CARRIED OUT IN DISCOVERY TO ASSESS THE PHARMACODYNAMIC SAFETY OF PROMISING NEW MOLECULES.

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The precious costs of time and effort realized by the failure of promising new molecules either in early to late development or after marketing authorization have motivated the pharmaceutical industry, regulatory authorities and the scientific community to seek ways of mitigating that risk and optimizing the chances of a successful and sustained registration of promising new drugs. De-risking includes not only addressing concerns related to toxicology (e.g., general, genetic and reproductive toxicology), but also toxicity resulting from the acute pharmacodynamic activity of the potential clinical lead candidate. The approach to pharmacodynamic safety testing is multifaceted beginning with an understanding of the pharmacodynamic properties of a molecule including: the effects of normal and exaggerated activity at a therapeutic target, its off-target characteristics, the ability of a molecule to form potential toxic metabolites, and data from other in vitro and in vivo studies suggestive of a concern for adverse pharmacodynamic effects. A directed study of pharmacodynamic toxicity will lead to additional critical data which collectively with the other information will potentiate a compound’s potential for failure in development due to unwanted adverse findings. Amongst these potential adverse events associated with acute pharmacodynamic toxicity, those of the cardiovascular, respiratory and central nervous systems are highlighted as having the greatest potential for serious adverse events. However, on a case by case basis, other organ targets such as the gastrointestinal, renal, and endocrine systems may be evaluated for undesired pharmacodynamic activity. Finally, integration of all safety data, including the potential for pharmacodynamic toxicity, serves as a basis for judging whether a promising new molecule may successfully advance through clinical development and achieve a sustained presence in the marketplace for the treatment of devastating diseases for which new therapies are critically needed.

W 1377 MANAGING RESOURCE LIMITATIONS IN DISCOVERY TOXICOLOGY: INTEGRATION OF RISK MITIGATION APPROACHES INTO EFFICACY STUDIES AND OTHER STRATEGIES FOR NOVEL THERAPEUTIC TARGETS.

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Capital, personnel, consumables, and active drug are key resource limitations in discovery research targeted to reduce compound attrition. The needs for these limited resources frequently compete with those of drug development and pharmacology, which may be considered higher priorities. Superimposed on these resource limitations are critical time constraints for drug candidate progression. Several strategies may be applied to addressing these potential limitations. Integration of safety endpoints into discovery proof-of-concept studies provides an opportunity to identify on- and off-target effects well prior to the conduct of formal toxicology studies. However, these models are generally poorly characterized from a toxicological perspective. Strategies that focus on limited resources on those most important causes of attrition or specific studies to address known lead issues in backups may be applied without significantly increasing risk of attrition, and consideration should be given to balancing cost and quality of studies for providing minimally informed early-to-late changes. This presentation will focus on presenting an appraisal of the best approaches to efficiently and economically generate interpretable safety data in discovery, given the constraints of toxicologists and pathologists charged with providing risk assessment to compounds soon to enter Development.

W 1378 STRUCTURE ACTIVITY TOXICOLOGY (SAT) AS A MEANS OF DE-RISKING COMPOUND FAILURE.

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Structure activity relationships are applied to identifying the best lead candidates that have affinity for the therapeutic target site and possess desirable properties conducive to advancement into development. However, a structural series can also be fraught with potential for toxicity of the core structure or a metabolite and much is known of specific structural moieties that pose risk of clinical safety. This area of study is referred to Structure Activity Toxicology and represents a holistic approach to safety science. The presentation will include strategies of de-risking molecules with potential adverse structural moieties and metabolites and successful means of risk mitigation that have been applied in the course of identifying promising clinical lead candidates.

W 1379 MITIGATION STRATEGIES DURING DISCOVERY LEAD OPTIMIZATION: MANAGEMENT OF A PRECLINICAL OFF-TARGET ADRENAL FINDING.

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Compound safety studies conducted in rats and dogs during Lead Optimization resulted in an off-target dose dependent adrenal finding, characterized by increased cortical vacuolation and adrenal weights. The finding did not appear to be development limiting, but the absence of routine biomarkers for detection of adrenal toxicity raised concerns. A team was created to develop and execute a plan to further characterize this finding, understand the cause, and address the risk of bringing the compound into clinical trials. The underlying hypothesis was that the compound inhibited the adrenal steroidogenesis pathway, resulting in accumulation of intermediate metabolites. Lipid stains (ORO and adiopophilin) of adrenals of compound treated animals revealed neutral lipids within the vacuoles. By electron microscopy, membrane bound cytoplasmic vacuoles appeared empty or contained variably granular to lucent substance, with acicular clefs and foci of membranous material. There was no evidence of cytotoxicity. In rat, dog and human adrenal microsomal assays and human H295R adrenal carcinoma cell line cultures, treatment with compound or a positional isomer partially inhibited the 21-hydroxylase mediated metabolism of progesterone to 11-deoxycorticosterone. Addition of the isomer
to a bovine adrenal cortical epithelial cell (BACC) line devoid of CYP21 mRNA expression still induced lipid vacuolation in the absence of cytotoxicity. Resulting gene expression analysis of BACC mRNA demonstrated increased expression of the ACTH receptor MC2R and steriodogenic acute regulatory protein (StAR). Moreover, the isomer weakly inhibited liver-X-receptor (LXR) pathways in functional assays and target gene analysis. Therefore, the mechanism of compound-induced increased adrenal lipid vacuolation appears to involve the steriodogenesis pathway and, possibly, the LXR pathway. The results indicate that the adrenal finding is an adaptive change and may be monitorable in clinical trials using an ACTH stimulation assay.

1380 HIGH-THROUGHPUT COMPUTATIONAL SCREENING FOR PLAUSIBLE GENE-ENVIRONMENT INTERACTIONS IN AUTISM.

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Autism Spectrum Disorder (ASD) is a pervasive neurodevelopmental condition affecting many in industrial nations. Although the causes of ASD are widely debated, it has been increasingly linked to gene-environment interactions. So far, no clear causative marker has been identified, and as a result no directed search for a plausible gene or environmental trigger can be undertaken. We pursued a high-throughput computational screening (HTS) approach targeted at gene products, in which hazardous etiologic agents (ligands) and suspected biological targets (proteins) can be massively matched to each other. To date, an in-house computational docking protocol has been developed and tested using tryptophan hydroxylase (TPH). TPH was docked against a small library of test compounds, including known substrates/inhibitors as a validation set. We found several chemical structures with docking scores consistently similar or better than of tryptophan, which is the natural substrate of TPH. It suggests that these chemicals may have a potential to interfere with TPH-related neurotransmitter pathways. The suspected candidates include folic acid, ochratoxin A and several organophosphates. Variations (up to 50%) in protein/chemical pairing were observed across natural tissue-specific TPH isoforms, as well as their minor types: r41274348, r41274350 and others. The developed docking protocol is currently employed in screening compounds from the ATSDR HazDat database. Other large chemical screening libraries of developmental toxins, food additives and drugs, household products, occupational hazards, and pesticides are being developed. Further, the HTS technology will be applied to 25 selected candidate genes products, along with their minor types, and then, subject of available computational resource, to an exhaustive set of targets form neurological pathways. By results of these studies, a combinatorial number of possible gene-environment interactions that could be implicated in autism is expected to drop to the size practical for in-depth laboratory and epidemiologic investigations.

1381 MODELING THE ESTERASE DOMAIN OF NEUROPATHY TARGET ESTERASE (NTE).

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NTE is a neuronal integral membrane lysophospholipase of unknown structure that is implicated as the target of neuropathic organophosphorous compounds (OPs). These OPs represent a class of compounds that induce a delayed axonopathy termed OP-induced delayed neurotoxicity (OPIDN). Obtaining an experimentally derived three-dimensional structure for NTE would provide further insights into its physiological and pathological roles. However, due in part to its membrane association, crystallization of NTE and its esterase domain (termed NEST) has yet to yield acceptable results. In the interim, molecular modeling can be undertaken to elucidate the putative structural organization of NTE’s protein domains. Previously, we showed that the NTE catalytic domain (a subset of its esterase domain) is homologous to the plant protein patatin and developed a corresponding homology model. We have now extended this model based on the crystal structure of human cytosolic phospholipase A2 (cPLA2; PDB ID 1cjy). Sequence alignments between NEST and cPLA2 indicated that these sequences share 16% identity. The NEST model was built via virtual mutagenesis of the tem- plate cPLA2 structure based on the sequence alignment obtained from the INUB server. The resulting model was refined by simulated annealing via heating the structure in silico to 500 K followed by a slow cooling in 25 K increments to room temperature. The packing of residues in the final model was assessed visually in PyMOL and revealed that NEST consists of an αβ-hydrolase fold with the active site Ser966 located on a flexible ‘nucleophilic elbow’ that is characteristic of serine hydrolases. In addition to this, the model recapitulated our previous findings that NTE is a neuronal integral membrane lysophospholipase of unknown structure

1382 PREDICTIVE SIGNATURES OF DEVELOPMENTAL TOXICITY MODELED WITH HTS DATA FROM TOXCAST™ BIOACTIVITY PROFILES.

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1383 A BBDR-HPT AXIS MODEL FOR THE PREGNANT RAT AND FETUS: EVALUATION OF IODIDE DEFICIENCY.

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A biologically based dose response (BBDR) model for the hypothalamic-pituitary-thyroid (HPT) axis for the pregnant rat and fetus is being developed to advance understanding of thyroid hormone disruptions and developmental neurotoxicity (DNT). The model for the pregnant rat and fetus quantify the compensatory mechanisms that govern the relationships between serum and brain thyroid hormone concentrations in the pregnant dam and fetus recognizing that these relationships may be affected by the mechanism of inhibition of different compounds. Initially, the model will be used to delineate perturbations in the HPT axis caused by inadequate dietary iodide. Later, environmental toxicants that alter HPT homeostasis will be examined. The current model uses the McLamahan et al. (2009) BBDR-HPT axis model for the pregnant rat as a foundation, but includes several new features: 1) formation rates of thyroid hormones, 2) negative feedback loop controlled by model-predicted brain concentrations of T3 and T4 that modulate the HPT axis, 3) thyroid hormone concentrations in the fetal compartment that are influenced by compensation, and 4) serum protein binding of thyroid hormones. Algebraic equations were developed to describe many physiologic and biochemical changes in the fetus and the dam. Calibration of the BBDR-HPT axis model will predict perturbations in the HPT axis caused by iodide deficiency, and ascertain the fetal HPT axis tolerance to maternal iodide deficiency before and after the HPT axis is functional in the fetus (GD 17.5). Support for this model was provided by a Cooperative Agreement R832134 and AFRL through the Henry Jackson Foundation for the Advancement of Military Medicine Contract 185137. This abstract does not necessarily reflect EPA policy.

1384 DEVELOPMENT OF A RAT GESTATION PBPK MODEL FOR PFOA/PFOA.


Perfluorooalkyl acids (PFAA) have a wide spectrum of consumer and industrial uses. Due to the strength of the C-F bond, these compounds are stable and resistant to biodegradation and metabolism. Two C8 PFAAs, perfluorooctanoic acid (PFOA)
and perfluorooctane sulfonate (PFOS), are found commonly in wildlife and the general population. Toxicity studies in experimental animals have raised concern about potential developmental effects of PFOA and PFOS in human populations. We developed PBPK models for these compounds to understand how the physiological changes that occur during gestation affect the tissue distribution of PFOA and PFOS in the mother and fetus. The gestation model expands upon a PBPK model for PFOA and PFOS in the adult female rat. In order to properly describe available data, our model required renal resorption, saturable binding in liver, and a varying free fraction of chemical in plasma. The model was used to simulate time course concentration data in maternal and fetal tissues during gestation from one study with PFOA and two studies with PFOS. The same model structure and same set of chemical parameters used in the adult rat model allowed for simulation of the tissue concentrations in the pregnant rat, indicating that pharmacokinetics in pregnancy are similar to those in the adult. Simple diffusion was sufficient to describe placental concentrations of PFOA and the transfer of both PFOA and PFOS to the fetus. Saturable binding in liver was not required for accurately simulating PFAA concentrations in fetal liver; apparently, fetal liver does not sequester PFAAs in a manner similar to adults. Gestation models provide initial values for tissue levels of PFAAs for simulating lactation exposure. The rat model has helped in addressing research needs for a more detailed model, such as identification of transporters responsible for renal resorption, with the eventual goal of extrapolating the model to humans to determine how the physiological changes and growth that occur during gestation in mother and fetus affect the pharmacokinetic behavior of PFAAs.

1385 COMPARISON OF SULFURYL FLUORIDE PHARMACOKINETICS ACROSS GENDER AND GESTATION IN RATS USING PBPK MODELING.

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Sulfuryl fluoride (SO2F2) is a structural and post-harvest fumigant used to control a wide variety of insects. SO2F2 is a key alternative to methyl bromide, an ozone depleting substance scheduled for phoucass by the Montreal Protocol. The potential systemic toxicity of SO2F2 depends upon the pharmacokinetics of the parent and/or its major metabolites, fluorosulfate (FS) and fluoride (F). Non-pregnant and late gestational PBPK models were developed to describe parent and metabolite pharmacokinetics following inhalation exposures at environmentally relevant levels. Metabolic rates for the hydrolysis of SO2F2 and FS in the blood measured in vitro were extrapolated and resulted in good fits to the in vivo plasma, urine, and tissue data. The conversion of SO2F2 to its metabolites is so rapid that the parent compound cannot be detected in blood or tissues, even immediately after 300 ppm exposures. The model supports the lack of measurable parent SO2F2, predicting blood concentrations below detection limits (<0.9 pmol/L) within seconds of cessation of exposure. Since bone is the primary depot of sequestration of F, the fluoride submodel includes surface and deep bone. The rate of exchange of F between surface and deep bone compartments is an important determinant of plasma and tissue fluoride concentration. The urinary elimination of FS differs between male and pregnant female rats; the model predicts a 10× lower rate in female rats. This difference is consistent with gender-specific urinary elimination of the analogous compound, sulfate. All other pharmacokinetic parameters are consistent between pregnant and nonpregnant rats and the overall pharmacokinetics of SO2F2 is consistent in pregnant and nonpregnant rats. This model will be useful in risk assessments that require estimates of tissue dosimetry for sulfuryl fluoride and metabolites in animal studies and in potentially exposed humans.

1386 PLASMA-BINDING PARAMETERS IN THE EMBRYONIC COMPARTMENT: AN IN SILICO SCREENING TOOL FOR ALPHA-FETOPROTEIN.

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Human alpha-fetoprotein (AFP) is perhaps one of the most studied proteins with a paucity of data related to its tertiary structure and functional interactions with ligands. As the primary embryonic protein constituent of plasma, we ask what makes AFP a member of the albumin family, so unique and structurally elusive? A homology model of AFP was developed using its primary sequence and the human serum albumin (HSA) crystal structure as a scaffold. In addition, ligand-specific affinity data for embryonic vs. adult plasma (AFP vs. HSA) constituents was culled from the literature. These models were used to analyze the role of changing gestational
DOTC had no effects. These results were in agreement with conventional neurotoxicity endpoints like brain region weight and size, but far fewer animals were needed for MRI. Regarding PET, DOTC and TBTO both altered the slope of normal developmental cerebral 18F-FDG uptake (PN 18, 22, 35, 62). For TBTO, however, the changes persisted through adulthood of the offspring. Results on gene expression –although preliminary- indicated differences between the two different brain regions, and gene expression patterns differed between PN22 and PN70, suggesting that neural biochemical changes concur with development. Further analysis is on going to further correlate conventional endpoints to the toxicology innovations like PET, MRI and gene expression.

### 1389 QUANTITATIVE ASSESSMENT OF NEURITE OUTGROWTH IN HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS USING AUTOMATED HIGH-CONTENT IMAGE ANALYSIS

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During development neurons undergo a number of morphological changes including neurite outgrowth from the cell body. Exposure to neurotoxins that interfere with this process may result in permanent deficits in nervous system function. While many studies have used rodent primary neural cultures, and human and non-human clonal cell lines to investigate mechanisms regulating neurite outgrowth and examine chemical effects on this process, few have used primary neurons of human origin. This study characterizes the molecular phenotype of human embryonic stem cell (hESC)-derived neural cells (hn23Tm) and uses automated high-content image analysis to measure neurite outgrowth in vitro. At 24 h after plating hn23Tm cells expressed a number of proteins indicative of a neuronal phenotype, including nestin, βIII-tubulin, microtubule-associated protein 2 and phosphorylated neurofilaments. Neurite outgrowth in hn23Tm cells proceeded rapidly, with a majority of cells extending one to three neurites of 40-60 μm in length by 48 h. In addition dose-dependent decreases in neurite outgrowth and cell viability were observed following treatment of hn23Tm cells with either bisindolylmaleimide 1, U0126, lithium carbonate, sodium orthovanadate or brefeldin A, all of which have previously been shown to inhibit neurite outgrowth in rodent primary neural cultures. Overall, the molecular phenotype, rate of neurite outgrowth and sensitivity of hn23Tm cells to neurite outgrowth inhibitors were comparable to other in vitro models. hn23Tm cells provide a model for assessing chemical effects on neurite outgrowth in the context of human biology and provide an alternative to the use of primary rodent neural cultures or clonal cell lines. This abstract does not necessarily reflect U.S. EPA policy.

### 1390 ACUTE INTOXICATION WITH MPTP ALTERS LOCOMOTOR ACTIVITY IN LARVAL ZEBRAFISH.

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To develop a rapid in vivo screen for EPA’s prioritization of toxic chemicals, we are characterizing the locomotor activity of zebrafish (Danio rerio) larvae after exposure to prototypic drugs that act on the central nervous system. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxicant that destroys dopaminergic neurons, causing parkinsonian signs in mammals that include abnormalities in locomotor activity. Studies with zebrafish in our laboratory have shown that developmental exposures to MPTP cause a biphasic dose-response pattern in locomotion. Because these results differed from previous findings in both zebrafish and mammals, it was important to identify the dose-response profile of pharmacological effects as well. We hypothesized acute exposure of zebrafish larvae to MPTP would produce locomotor changes, separate from those produced by developmental exposures. Zebrafish were raised in a 96-well microtiter plate at 26°C under a 14:10 hr light/dark period of light using video tracking. Acute exposure to MPTP yielded a biphasic dose-response function, with lower doses (0.8 and 1.6 μM) during alternating periods of light and dark (infrared) using video tracking. These results differed from the changes in locomotor activity due to developmental exposures at the same doses, where hyperactivity was seen at intermediate doses (1.6-12.5 μM) and moderate hypoactivity was seen only at the highest dose (50 μM). Therefore, acute exposure to MPTP results in changes in locomotor activity that are distinguishable from the effects of developmental exposures. (T.D. Irons is supported by NIH NIGMS Initiative for Maximizing Student Diversity and NIEHS National Research Service Award (T32 ES007126). This is an abstract of a proposed presentation, and does not necessarily reflect EPA policy.)

### 1391 CHLORPYRIFOS DISRUPTS NEUROLIGIN-MEDIATED SYNAPSE FORMATION.

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The causes of autism spectrum disorder (ASD) are poorly understood. While genetic factors are important in determining susceptibility, there is evidence that environmental factors also interact with synaptic formation in the developing brain. This hypothesis is derived from observations that 1) mutations in the gene that encodes neuroligin have been linked to ASD; 2) increasing evidence suggests that the patterns of synaptic connections between neurons are altered in the brains of autistic children; and 3) the OP chlorpyrifos and neurexin ligands have both been shown to independently influence the formation of these connections. Additional reasons to suspect that OPs may contribute to determining the risk for ASD include documented widespread exposure of pregnant women and children to OPs in both agricultural and residential settings as well as findings from recent human studies suggesting that perinatal exposure to OPs may be linked to increased risk for ASD. Using embryonic hippocampal neurons co-cultured with COS-7 cells transfected with neuroligin, we show that chlorpyrifos disrupts neuroligin-mediated synapse formation in vitro in the absence of effects on cell viability and neuroligin expression. These data support our hypothesis and provide a biological mechanism to support the epidemiological data linking OP exposure to increased risk for ASD. This work was supported by the UC Davis M.I.N.D. Institute.

### 1392 DEVELOPMENTAL BEHAVIORAL TOXICITY OF BISPHENOL A: DEFINING THE ROLE OF ESTROGEN RELATED RECEPTOR GAMMA.

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The use of bisphenol A (BPA) to produce polycarbonate and resin-lined food containers has resulted in detectable BPA in serum and breast milk of pregnant and nursing women throughout the US population. Evidence from rodent studies indicates that exposure to low levels of BPA during critical stages of central nervous system (CNS) development can produce persistent behavioral impairments. The mechanism by which low dose BPA exposure impairs CNS development is unclear, although the predominant assumption is that BPA acts as an estrogen receptor (ER) ligand. However, BPA’s binding affinity for ER is 10,000-fold less than the natural ligand estradiol. A more likely endogenous target is estrogen related receptor gamma (ERRγ), to which BPA binds with 100 to 10,000-fold greater affinity than ER. Zebrafish embryos were exposed to BPA concentrations ranging from 0.001 μM to 100 μM from 0 to 5 days post fertilization (dpf). Exposure concentrations <20 μM resulted in no observed adverse morphological effects, whereas higher concentrations reduced heart rates and produced pericardial and yolk sac edema. Next, zebrafish were developmentally exposed to low BPA concentrations (±10 μM) and behavior endpoints were assessed at 6, 45, and 75 dpf. Low concentrations (0.01 μM) to moderate dose activity in all behavior tests. The role of ERRγ in mediating BPA-induced behavioral toxicity, an ERRγ-antagonist Src inhibitor, was effective in blocking the behavioral effects of BPA exposure. These results were supported in part by NIEHS training grant #T32ES07060 and NIH grant #ES00210.

### 1393 ARSENIC INHIBITS NEURITE OUTGROWTH BY INHIBITING LKB1-AMPK SIGNALING PATHWAY.

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Background: Arsenic is an environmental pollutant inducing numerous pathological effects, including neurodevelopmental disorders. Objectives and Methods: We used Neuro-2a neuroblastoma cells as a model of developing neurons and evaluated the role of LKB1-AMPK pathway in arsenic-induced developmental neurotoxicity. Results: Addition of low concentrations of arsenic (±5 μM) during differentiation...
caused an inhibitory effect on the neurite outgrowth in Neuro-2a cells in the absence of cell death. AMPK activation induced by retinoic acid exerted their toxicity by different effects (e.g. ROS generation, ion-release, loading of macrophages) and hence their lung toxicity is determined by different material properties.

We propose to use mass concentration as dosimetry for all particles. At the same time we stress the necessity to fully characterize the tested materials, this will allow for transformation of mass to other dosimetry parameters. The correct dosimetry can only be identified by recognizing the very material properties causing the toxic effect.

1396 OCCUPATIONAL RISK ASSESSMENT OF MULTIWALL CARBON NANOTUBES.

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Multi Wall Carbon Nanotubes (MWCNT) are of great commercial interest, as they combine high electrical conductivity, good mechanical strength and excellent thermal conductivity. It is therefore essential to ensure a sustainable development of these materials. This requires a risk assessment of production and use of MWCNT followed by risk management measures. The potential exposure of workers via inhalation during production, processing and handling of MWCNT NC 7000 from Nanocyl has been measured. Several methods were used such as EPI, CPC, SMPS, etc. A new detector (Nano-ID) combining a cascade impactor and a diffusion deposition able to identify particles from a few nm up to 30 μm was also used. In addition, in-situ on-line MWCNT specific counter based upon Nano-ID was employed. Measurements have been carried out by Nanocyl and several organizations at Nanocyl facilities. Measurements consistently demonstrated that exposure can be minimized by applying appropriate handling measures. For hazard assessment, a 5-day and a 90-day inhalation toxicity study with NC 7000 was performed according to OECD test guideline 413. Rats inhaled concentrations of 0 (control), 0.1, 0.5, or 2.5 mg/m³ on 6 h/day on 5 days/week. The exposure resulted in no systemic toxicity, but a concentration-dependent increase of lung weights and granulomatous inflammation with histiocytic, neurophilic inflammation and intra-alveolar lipoproteinosis in the lung and lung-associated lymph nodes at 0.5 and 2.5 mg/m³; at 0.1 mg/m³, there was still minimal granulomatous inflammation. The data were analyzed by the Uncertainty Factor Method and the Benchmark Dose approach. The risk assessment indicates that (i) toxicological data appear to be sufficient for initial risk estimates for non-cancer lung responses, (ii) standard methods for hazard and risk assessment appear feasible and (iii) strict industrial hygiene measures during handling and processing are effective.

1395 INHALATION TOXICITY STUDIES WITH 12 NANOMATERIALS USING DIFFERENT DOSIMETRIES–NONE FITTED ALL.

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Several studies demonstrate that identical mass concentrations of nano-particles are more toxic to the lung than larger particles of similar chemistry. Surface area and particle count concentration have been discussed as critical factor responsible for higher pulmonary toxicity of nanoparticles (Oberdoerster et al. 2005 EHP 113, 825-839). We tested the toxicity of 15 substances (12 nanomaterials: SiO2, surface coated SiO2, TiO2 P25, coated TiO2, CeO2, coated CeO2, ZrO2, BaSO4, carbon black, MWCNT1, MWCNT2 and – for comparison – 3 micron-sized materials: quartz, TiO2, ZnO) after inhalation exposure. All materials were sufficiently characterized and tested by the well-established short-term inhalation toxicity protocol for nanomaterials (Ma-Hock et al. 2009, Inhal. Toxicol. 21, 102); total protein concentration and polymorphonuclear neutrophils in bronchoalveolar lavage fluids were used as indicators of effects in the lung. The observed lung toxicity was related to (a) particle mass, (b) particle volume, (c) particle surface area and (d) particle count per aerosol volume (aerosol concentration) and per lung surface (lung burden).

For insoluble nanomaterials the lung toxicity varied over two orders of magnitude when using mass concentration as dosimetry, and between different materials toxicity was largely independent of surface area, particle count, volume or mass. Evidently, there is not one dosimetry for all nanomaterials. Rather, nanomaterials exert their toxicity by different effects (e.g. ROS generation, ion-release, loading of macrophages) and hence their lung toxicity is determined by different material properties.

We propose to use mass concentration as dosimetry for all particles. At the same time we stress the necessity to fully characterize the tested materials, this will allow for transformation of mass to other dosimetry parameters. The correct dosimetry can only be identified by recognizing the very material properties causing the toxic effect.

1397 MESOTHELIOMA INDUCTION BY MICROMETER-SIZED MULTI-WALL CARBON NANOTUBE INTRAPERITONEALLY INJECTED TO P53 HETEROZYGOUS MICE.

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Nanomaterials of carbon origin tend to form various shapes of particles and aggregates in micrometer dimensions. Among them, certain make of multi-wall carbon nanotubes (MWCNT) forms fibrous or red-shaped particles of length around 10 to 20 micrometers with an aspect ratio of more than three. Fibrous particles of this dimension including asbestosis and some man-made fibers are reported to be carcinogenic, typically inducing mesothelioma. Here we report that such micrometer-sized MWCNT induces mesothelioma when administered intraperitoneally to p53 heterozygous mice that have been reported to be sensitive to asbestos. In this study, as an accidental finding, fullerenes which was administered intraperitoneally as negative control of the study turned out to be distributed systemically. It was histologically suggested that the surface of fullerenes aggregates were corroded by phagocytic activities, possibly down to nanometer dimensions and brought out from the peritoneal cavity. Our results indicate that newly made particulate matters of various sizes have a potential to exhibit known as well as totally unknown toxicity, especially of chronic nature. To maintain sound activity of industrialization of nanomaterials, timely transfer of information on the chronic toxicity of the new materials or the products to the manufacturers would be of great importance. In order to facilitate such process, cultivation of proper toxicology scientists who are able to predict such “new” chronic toxicity by making full use of traditional as well as recent methodologies should be promoted and supported not only by the government but also by the private sector.
PULMONARY TOXICITY OF CERIUM DIOXIDE PARTICLES IS MODULATED BY SIZE AND COATING: INHALATION STUDIES WITH NANO-, AGGREGATED NANO-, AND MICRON-SCALE PARTICLES AND PARTICLES WITH DIFFERENT SURFACE CHEMISTRY.

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Pulmonary toxicology studies in rats suggest that nano-particles are more toxic to the lungs than larger, micro-scale particles of similar chemical identity at identical mass concentrations (Oberdoerster et al. 2000, Res Rep. Health Eff. Inst. 96, 5-86, Stoeger et al. 2006, EHP 114, 328-333 Wittmaack 2007, EHP 115: 187-194). In this study we used CeO2 as model substances with different particle sizes, agglomeration states and surface chemistry designed for different applications. Male Wistar rats were head-neck exposed to test atmospheres of CeO2 for 6 hours on a day 5 days. Toxicity was assessed by examination of broncho-alveolar lavage fluid (BALF) shortly after the exposure and after a recovery period of 2 months or 3 weeks. Exposure to nano-scale CeO2 (spark generated) caused pronounced effects at a mass concentration of 0.14 mg/m3 (increased levels of ALP, GGT, protein, neutrophils and lymphocytes). Aggregates of CeO2 particles of similar size induced milder changes (ALP, GGT, protein and neutrophils) at more than 10 times higher mass concentration. Similar mass concentration of the micro-scale material failed to induce any measurable signs of toxicity. Agglomerates of unmodified CeO2 and alumina doped CeO2 (similar particle size and surface area) were administered at three concentrations to rats by inhalation exposure as described above. Both materials caused concentration-related changes of BALF parameters, increased lung weights and inflammation in the lung. However, the concentration-response relationship of the doped CeO2 was steeper than that of unmodified CeO2. It can be concluded that size as well as aggregation/agglomeration state and surface chemistry strongly influence the pulmonary toxicity of nanomaterials.

IN VIVO AND IN VITRO ASSESSMENTS OF MICRONUCLEUS INDUCTION BY AMORPHOUS SILICA PARTICLES.

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The development of a risk management system for nanoscale particle-types requires a base set of hazard data. These data could include screening-type genotoxicity studies. However, there is little agreement on the appropriate tests for evaluating genotoxicity responses to particulate materials. One objective of this study was to assess the induction of micronucleated reticulocytes in rats exposed to aerosolized amorphous silica (AS) nanoparticles. Male rats were exposed for 1 or 3 days, 6 h/d, to freshly generated AS nanoparticles (37 or 83 nm) at concentrations ranging from 3.1 x 10E7 to 1.8 x 10E8 particles/cm3. Control animals were sham-exposed to room air. Peripheral blood samples were collected 24 hours postexposure, fixed and analyzed by flow cytometry according to the In Vivo MicroFlow Plus Rat Micronucleus assay kit. Approximately 20,000 reticulocytes were analyzed per animal. For both particle sizes and exposures there were no statistically significant increases in micronucleated reticulocytes vs. controls. These data were compared with an in vitro assessment of another form of AS fine particles, using a Micronucleus assay kit in CHO/K1 cells. There were no significant increases in the percentage of micronucleated cells at any concentration tested, although cytotoxicity was observed at concentrations > or = 5.2 μg/cm2 (plate surface area) with a dose-dependent decrease in the percentage of cells in the G0/G1 cell cycle phase, along with a higher percentage in G2/M. A second objective was to determine whether identification of cytotoxicity endpoints correlated with genotoxicity parameters. Increased cytotoxicity (LDH and MTT assays) and inflammatory cytokine release (TNFα and IFNγ) were measured in macrophages (J774A.1 and rat alveolar epithelial (L2) and macrophage (N8383) cell lines. Studies are ongoing to develop appropriate in vivo and in vitro screening assays with fine-sized and/or nanoscale particles to detect genotoxicity.

ASSESSMENT OF PULMONARY TOXICITY FOLLOWING INTRATRACHEAL EXPOSURE TO SILICON NANOWIRES.

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Single-crystal silicon NW were synthesized by the vapor-liquid-solid method in an ultrahigh vacuum-chemical vapor deposition chamber with silane as the silicon precursor and gold as the catalyst (~20-30 nm Diameter x ~15 μm Length, with a 20 nm gold particle catalyst at one end). NW were injected in a physiologic dispersion medium (DM, phosphate-buffered saline + 0.6 mg/ml rat serum albumin + 0.01 mg/ml dipalmitoyl phosphorylcholine), and sonicated. On day 0, Sprague-Dawley rats were intratracheally-instilled with the NW in DM at a dose of 10, 25, 50, 100, or 250 μg or DM alone (control). Rats were humanely sacrificed 1, 3, and 7 days post-exposure and the right lung was lavaged. The lavage fluid and cells were analyzed for indicators of lung injury and inflammation. On days 1 and 3, there was a dose-dependent increase in lung injury, indicated by elevations in lactate dehydrogenase and albumin in lavage fluid. There was also a dose-dependent increase in inflammation indicated by the presence of neutrophils in the lung on day 1, which persisted on day 3 in the rats treated with the highest dose. In addition to neutrophils, there were also significant increases in alveolar macrophages, lymphocytes, and eosinophil influx into the lungs. Macrophage uptake of NW was observed in cells recovered at all time points and this uptake was paralleled by increased oxidant production in these cells. These initial lung injury and inflammatory responses resolved by day 7. To summarize, the NW were found to induce a transient lung injury and inflammation accompanied by cellular oxidant production. Studies are ongoing to assess long-term pulmonary responses to NW as well as to assess lung distribution and clearance of NW over time.

ASSESSMENTS OF CERIUM DIOXIDE (CeO2) PARTICLES USING AN IN VIVO ASSESSMENT METHOD.

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Engineered nanoparticles as nanotechnology materials have been designed intentionally with the physicochemical characteristics for the specific application. In the industry field that relates the human to direct ingestion, engineered nanoparticles are applied to pharmaceuticals, foods, cosmetics, and so on. Among these engineered nanoparticles, it is feared that the exposure risk of fullerenes and their derivatives is more serious from the occupational and/or living environment through the oral, dermal and inhalation route by the rapid commercialization. However, the exposure to the human health and the biological behavior by the exposure have not studied sufficiently. In this study, we have examined the behavior of C60 fullerene after injection into the tail vein in rats. The C60 fullerene was extracted with toluene from...
tissues and then measured by LC/MS/MS. The fullerene was detected in liver, kidney, spleen and lung in all injected rats. The highest concentration was 64.5 μg/mg, corresponding to 0.52 mg/m3 Cd with MMAD 1.7–2.9, GSD 6.5–4.3) was generated. A total of 5.3 % of inhaled materials was found in the lung. Cd was found excreted mainly in feces immediately after exposure, but not in urine. The exposure to Cd/SO2/OH2 QDs results in mild reactions in animals’ lungs indicated by increased lung weights (+16 %) and increased neutrophil count in BALF. The clearance of QD from the lung appeared to be low. No substantial translocation was observed.

**1403 SHORT-TERM INHALATION TOXICITY STUDY WITH CD-BASED QUANTUM DOTS.**

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Quantum Dots (QDs) are a new class of metal-based nanoscaled fluorescent particles. Their crucial physicochemical properties offer several advantages compared to other dyes, QDs are of great interest for histological, cellular and biomolecular imaging. The possibility to coat QDs with molecules for drug delivery, antibodies or tumor-targeting ligands also opens new opportunities in medical applications. However, little is known about the toxicological behavior of this material. We performed a short term (5 day) inhalation study to investigate the pulmonary and systemic toxicity as well as the distribution of Cd-based QDs after inhalation exposure. Male Wistar rats were head-nose exposed to clean air and maximal technically attainable concentration of QD on 5 consecutive days for 6 hours respectively. Shortly after the last exposure and 3 weeks thereafter animals were sacrificed for gross necropsy, histological examination as well as examinations of bronchoalveolar lavage fluid (BALF). Highly respirable aerosol (0.07 mg/m3 CsCd/OH2 QDs corresponding to 0.52 mg/m3 Cd with MMAD 1.7–2.9, GSD 6.5–4.3) was generated. A total of 5.3 % of inhaled materials was found in the lung. Cd was found excreted mainly in feces immediately after exposure, but not in urine. The exposure to Cd/SO2/OH2 QDs results in mild reactions in animals’ lungs indicated by increased lung weights (+16 %) and increased neutrophil count in BALF. The clearance of QD from the lung appeared to be low. No substantial translocation was observed.

**1404 AN IN VITRO ASSAY FOR THE PREDICTION OF CYTOKINE RELEASE SYNDROME.**

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Acute cytokine release syndromes (CRS) are associated with some therapeutic antibodies in man, leading to a spectrum of clinical signs from nausea to serious hypotension and tachycardia. When anticipated this syndrome is typically manageable, however this adverse reaction recently became headline news when a massive and unexpected CRS occurred within a few hours of dosing six healthy volunteers with a therapeutic antibody (TGN1412) putting their lives at risk due to multiple organ failure. There are two potential mechanisms of antibody-induced cellular activation Type I which is dependent on the ligation of antigen by the V region of the antibody + Type II which is dependent on the ligation of Fc receptors by the Fc portion of the antibody. In this work we have developed and In vitro assay using isolated leukocytes that differentiates these two mechanisms and may be a useful test for predicting potential cytokine release syndrome. Briefly, isolated leukocytes are incubated with either immobilised (type 1) or free (type II) antibody, spun, the supernatants collected and measured for CRA signature cytokines via cytokine bead array. In order to put the cytokine release induced by any test antibody into context, well-characterised clinical antibodies are also included in the assay. In this way the cytokine release induced by a test antibody can be translated into probable clinical outcome. The control antibodies generate a combined cytokine release in the expected hierarchy, with IgG4 (neg con) being the lowest (usually <50pg/ml), followed by CD25 (approx 1000ng/ml) CD3 (approx 500ng/ml) or in combination with CD28 (approx 10,000ng/ml). The developed assay has intra and inter assay CV of <30%, dilutional linearity of 0.99-0.93 (depending on analyte), is highly specific and has sample stability of at least 6 months. Therefore this assay represents a useful tool for the possible prediction of CRS in man.

**1405 USE OF THP-1 CELLS TO IDENTIFY PROHAPTENS.**

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Animal based testing is currently used for screening of potential allergenic chemicals. Recent efforts have been directed at the development of non-animal based alternative tests for the identification of skin sensitizers including the use of in vitro cell activation assays. Upregulation of activation and costimulatory markers in anti-CD3 present cells are key events in the sensitization process and have been reported to serve as indicators of skin sensitization. Prohapten identification remains a limitation due to the lack of bioactivation of prohaphtens in these cell lines. The present study evaluated the efficacy of a human prohapten exposure in cell surface marker expression (CD86, CD54, CD44 and CD40) on THP-1 cells. Cells were exposed to the prohapten benzo(a)pyrene (BaP), 7,12-dimethylbenz(a)anthracene (DMBA) and carvone oxime (CVO) at concentrations ranging from 1-10 μM for 24 and 48 hours. The direct-binding hapten, dinitrochlorobenzene (DNCB), was used as a positive control. Bioactivation of prohaphtens was achieved by addition of a rat liver microsomal (S9) cocktail to the cell cultures. Flow cytometry data demonstrated a consistent dose-dependent increase of surface expression of the T-cell costimulatory molecule CD86 when cells were dosed with a hapten or prohapten in the presence of S9. Expression of the adhesion molecule CD54 (ICAM-1) and the antigen presenting cell costimulatory molecule CD40 were also significantly elevated in both hapten and prohapten (+ S9) treated cells, however S9 alone also upregulated CD54, CVO and DMBA, but not BaP, induced inconsistent increases in the adhesion molecule CD44. In conclusion, modification of in vitro cell culture assays to include co-incubation with microsomes enhances identification of prohaphtens and allows them to be clearly distinguished from haptens.

**1406 CYTOKINE LEVELS IN TISSUE AND MEDIUM OF PRECISION-CUT LUNG SLICES DURING PRODUCTION AND INCUBATION.**

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Precision-cut lung slices (PCLS) provide a multicellular, 3D tissue model from which biochemical and histological data can be extracted, compared, and analyzed. We have examined the PCLS cytokine response to slice production and incubation and the effect of anti-inflammatory or pro-inflammatory stimuli. Lungs from adult male Fisher 344 rats were removed and processed for slicing in cold N7 solution. Using the roller method, slices were cultured in a serum-free, M199-based-medium (± 0.1 ng/ml hydrocortisone) at 37°C with 5% CO2 for up to 6 days. Phortress at 100 μg/ml hydrocortisone (NSC710305), previously shown to elicit IL-1β and TNFα response in human PCLS, was added at day 0 (10 and 100 μM) and at days 1, 2, and 3 (100 μM) for 72 hrs. Medium and slices were collected every 24 hr, post-treatment. Cytokine levels were determined histologically correlated with cytokine data. To conclude, observing the temporal cytokine response in this in vitro model allows for the proper timing for the application of toxicants. PCLS data obtained prior to the diminution of initial cytokine surge may be confounded by cytokine levels generated by slice production. In this work we have developed and In vitro assay using isolated leukocytes that differentiates these two mechanisms and may be a useful test for predicting potential cytokine release syndrome. Briefly, isolated leukocytes are incubated with either immobilised (type 1) or free (type II) antibody, spun, the supernatants collected and measured for CRA signature cytokines via cytokine bead array. In order to put the cytokine release induced by any test antibody into context, well-characterised clinical antibodies are also included in the assay. In this way the cytokine release induced by a test antibody can be translated into probable clinical outcome. The control antibodies generate a combined cytokine release in the expected hierarchy, with IgG4 (neg con) being the lowest (usually <50pg/ml), followed by CD25 (approx 1000ng/ml) CD3 (approx 500ng/ml) or in combination with CD28 (approx 10,000ng/ml). The developed assay has intra and inter assay CV of <30%, dilutional linearity of 0.99-0.93 (depending on analyte), is highly specific and has sample stability of at least 6 months. Therefore this assay represents a useful tool for the possible prediction of CRS in man.

**1407 APPLICATION OF AN IN SILICO LIVER MODEL TO DETERMINE NUCLEAR RECEPTOR MEDIATED PATHWAYS IN LIVER CANCER.**


Nuclear receptors (NRs) are ligand-activated transcription factors that control diverse cellular processes. Chronic stimulation of some NRs in rodents can result in increased incidence of liver tumors. Tumors are thought to develop through non-genotoxic mechanisms with unclear relevance to humans. Human CAR, PXR,
CELULAR SYSTEMS BIOLOGY (CSB) ASSAY PANELS PROVIDE AN IMPROVED METHOD FOR PREDICTING HUMAN LIVER INJURY.

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Hepatotoxicity is the leading cause for late stage attrition of drugs and remains a concern for Pfizer’s portfolio. Whereas some compounds will show toxicity only when the dose is increased, it is the dose-independent, idiosyncratic type reactions that are unpredicted in Pfizer’s current animal models and are only apparent when large cohorts of patients are exposed. Attraction at this late stage is detrimental both for the patient and to Pfizer. Pfizer currently uses cryopreserved human hepatocytes and high content imaging to predict human hepatotoxicity (Xu et al., 2008). Cells are treated with compound at a concentration equal to 100x human cmax. Four parameters (ROS/MMP/Cell number/GSH content) are analyzed and compounds are considered positive if any of the parameters flag above a pre-defined threshold. The predictivity was found to be 50% for the 146 liver toxicants that were tested. However, human hepatocytes are difficult to obtain, expensive and vary greatly from donor to donor. Cellumen has developed two CSB assay panels and classifiers that predict in vivo rodent hepatotoxicity. The panels use fresh rat hepatocytes and HepG2 cells and measure 8 and 10 cellular functions (respectively) at 3 time points across a 10 point dose-response. Cells are easily obtained and quality is homogeneous and consistent. Pfizer tested 300 compounds consisting of liver toxicants, compounds toxic to other organs and non-toxic drugs in the CSB panels. 178 of these compounds were also in the original test set published by Xu et al. (ibid.). The Xu classifier successfully scored as positive 39% of the compounds known to cause human hepatotoxicity in this set, with no false positives. Using the data from Cellumen’s rat hepatocyte and HepG2 panels, we were able to achieve higher predictivity of human hepatotoxicity, correctly scoring 46% of the hepatotoxic compounds in this set, with no false positives.
Novel alternative methods for assessing DNT of chemicals and drugs are therefore needed. A short term zebrafish embryo assay might give an ethical acceptable, and cost-efficient test for numerous compounds.

We developed 2 complementary assays with zebrafish embryo and larva as models to evaluate the developmental neurotoxic potential of chemicals. In these assays locomotor activity was used as an endpoint: (i) spontaneous tail coiling of embryos at 24–26 hours post fertilization (hpf) and (ii) swimming behavior of larvae at 120 and 144 hpf. Embryos were exposed within 2 hpf to a compound at a concentration level which did not induce morphological abnormalities (Selderslagh et al., 2009, Repr. Tox.). By means of video footage and tracking software, we determined frequency and total duration of spontaneous tail coiling in embryos (egg stage) and analyzed swimming behavior of hatched larvae based on parameters such as distance moved, velocity and turn angle. Results from controls and exposed embryos or larvae were compared and based on differences in distribution of data obtained, effect percentages were determined for each concentration of the compound tested.

To assess whether the proposed assays based on locomotor activity were able to distinguish developmental neurotoxicians from negative compounds, we selected 6 positive (chlorpyrifos, chlorpromazine, methylmercury, methimazole, propyl thiouracil, thiamine mononitrate) and 4 negative compounds (saccharin, D-glucose, acetalaminophen, omeprazole). Analysis of the results of this intralaboratory validation study, revealed that these new methods combined, have a high predictive capacity and may potentially be integrated in a testing battery to screen for DNT, and anticipate to 3R.

IS 1413 IMPACT OF TUNGSTEN AND TUNGSTEN ALLOYS ON HEALTH RISK.

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Debate of the potential human health effects of tungsten (W) is fostered by widespread exposure to naturally occurring W in air, soil, water, and the diet and anthropogenic sources including the use of tungsten alloy (WA) in military munitions. There is particular concern about the exposure of military personnel to retained W-based munitions fragments. The cellular and molecular mechanisms of systemic W toxicity and the role of W speciation in W-induced toxicity remain poorly defined. Intensive research on the characterization of potential adverse health effects associated with tungsten exposure is underway and employs multiple routes of exposure including oral, inhalation and implantation. Other recent studies have characterized W transport mechanisms, pharmacokinetic parameters, and biochemical and pathological indices in vitro and in vivo. These efforts have identified new biomarkers of exposure and effect as well as new opportunities for therapeutic intervention or management of potential health hazards. This session will review current research programs as well as describe the recent studies examining the toxicity and carcinogenicity of embedded tungsten and heavy metal tungsten alloy pellets and refined corrosion assessments to define the degradation rate of the pellets. Our panel of experts will discuss the absorption/distribution, and elimination of tungsten and effects on the nervous system and immune system with particular emphasis on the mechanisms through which W may produce toxic effects.

IS 1414 THE 2009 TENNESSEE FLY ASH SPILL: AN ENVIRONMENTAL EMERGENCY CASE STUDY.

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On December 22, 2008, at approximately 1:00 AM, a retaining wall supporting a surface impoundment of fly ash sludge at the Kingston Fossil Plant in Harriman, Tennessee, breached releasing an estimated 5.4 million cubic yards of material into the Emory and Clinch Rivers and surrounding areas. The release extended over approximately 300 acres of land outside of the containment site. A wave of ash and water destroyed homes, killed electrical generation equipment, covered roads and rail tracks, and necessitated the evacuation of nearby residents. Responders at the scene pursued a variety of activities intended to assess the extent of both the release and the potential hazard posed by the event and to contain the spread of any hazardous materials released into the environment. The roles, responsibilities, and interactions of various local, state, and federal partners present at the scene had a substantial impact on response activities. This included oversight of the development and initiation of a large program of environmental sampling of the air, soil, and water followed by analysis of the resulting data. The Tennessee fly ash spill is representative of other environmental emergencies, and is therefore an excellent case study in which to provide a framework for discussions concerning the role of toxicology in protecting environmental and human health in affected communities, and in determining the appropriate roles and actions of the various regulators at the scene.

Further exploration will allow us to focus on the mechanisms behind gender differences in cation transporter expression and function and protein translation differently in males and females which will have a major impact on the toxicological response. Both mechanisms and relevant examples of gender-dependent toxicities will be provided. To fully understand these issues, an overview will be provided that will allow participants to review recent findings on the divergence of gene expression between males and females in response to toxic insults influenced by gender specific drug elimination and cellular efflux. Elegant studies that demonstrate sex and growth hormonal dependence reveal the importance of these factors in toxic and therapeutic responses. Further exploration will allow us to focus on the mechanisms behind gender differences in carbox transporter expression in the GI tract and kidney. Interplay between gender and the underlying nutritional status of zinc, iron, and calcium, as well as the influence of transporter expression and toxicity. Essential element deficiencies result in gender specific up-regulation of transporters, thereby facilitating the transport of toxic metals such as lead and cadmium. Adequate focus will be provided on the immune system and how steroid hormones influence immunomodulatory proteins of the toll-like receptor family. These findings have relevance not only to the toxic response, but also to the pathogenesis and severity of infectious disease influenced by concurrent toxin exposure. Final gender differences in gene expression in the heart during cancer therapy will be addressed and how it affects signal transduction pathways controlling mitochondrial function and protein translation differently in males and females which will explain why females are better protected from the cardiotoxic effects of the chemotherapy.

S 1415 CAREER ALTERNATIVES IN TOXICOLOGY: LESSONS LEARNED.

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For individuals who desire to take a career break or those set to retire, many options are available. There are many avenues to explore including those that involve technical opportunities for toxicologists and environmental scientists. Of the many opportunities to explore, the Peace Corps and U.N. volunteer programs offer a myriad of opportunities for environmental scientists wishing to practice their trade abroad. In addition to these two examples, other alternatives will be discussed including internship and other work available in academia, which provides its own set of unique experiences. For example, just how does one go about leaving a career in cancer research and epigenetic toxicology to become an administrator at the Radiation Effects Research Foundation in Hiroshima, Japan? There are many positive sides to such a decision, including work on a historic project in a foreign country and interactions with scientists who may benefit from your insight; however, there can be disadvantages as well. Experienced panel members will highlight the “price-paid” for such decisions. What about options other than academic research, such as toxicologists with innovative ideas who wish to capitalize on their talents and drive by starting a biotechnology company? Our panel of experts will provide insight and tips on the challenges involved in bringing an idea for a commercial product to the market place. This specific discussion will note the distinct advantages and disadvantages of embarking on a career change from academia to establishing a biotechnology company. This last discussion will highlight the specific and unique challenges of starting a company, including acquisition of intellectual property rights, obtaining funding, and marketing of products. This session should be of interest to anyone looking to explore career alternatives off the beaten path.

S 1416 GENDER DIVERGENT XENOBIOTIC RESPONSES.

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Differences in exposure, anatomy, physiology, biochemistry, and behavior between males and females dramatically affect the biological response; yet most toxicologists have not received adequate attention in toxicology. This session will highlight cutting edge discoveries within gender divergent biology that have a major impact on the toxicological response. Both mechanisms and relevant examples of gender-dependent toxicities will be provided. To fully understand these issues, an overview will be provided that will allow participants to review recent findings on the gender differences in expression between males and females in response to toxic insults influenced by gender specific drug elimination and cellular efflux. Elegant studies that demonstrate sex and growth hormonal dependence reveal the importance of these factors in toxic and therapeutic responses. Further exploration will allow us to focus on the mechanisms behind gender differences in cation transporter expression in the GI tract and kidney. Interplay between gender and the underlying nutritional status of zinc, iron, and calcium, as well as the influence of transporter expression and toxicity. Essential element deficiencies result in gender specific up-regulation of transporters, thereby facilitating the transport of toxic metals such as lead and cadmium. Adequate focus will be provided on the immune system and how steroid hormones influence immunomodulatory proteins of the toll-like receptor family. These findings have relevance not only to the toxic response, but also to the pathogenesis and severity of infectious disease influenced by concurrent toxin exposure. Finally, gender divergence in gene expression in the heart during cancer therapy will be addressed and how it affects signal transduction pathways controlling mitochondrial function and protein translation differently in males and females which will explain why females are better protected from the cardiotoxic effects of the chemotherapy.
S 1417 MECHANISM OF GENDER-DIVERGENT EXPRESSION OF PHASE II ENZYMES AND MULTIDRUG RESISTANCE (MDR) TRANSPORTERS-IMPLICATIONS TO TOXICOLOGY.

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This presentation will review the latest findings of gender differences in two important systems affecting the toxicological response: phase II enzymes and multidrug resistance (MDr) transporters. UDP-glucuronosyltransferases (UGTs) catalyze the addition of glucuronic acid to endogenous compounds and xenobiotics, increasing hydrophilicity and enhancing elimination. Gender-divergent glucuronidation rates are observed in humans and rats, and mechanisms for expression differences will be discussed. Secondly, multidrug resistance (MDr) transporters are ATP-biding cassette transporters that efflux amphipathic cations from cells and protect tissues from xenobiotics. Unfortunately, Mdr transporters also efflux anticancer drugs from tumor cells, resulting in multidrug resistance. The latest findings on the role of gender on regulation of Mdr genes in vivo will be discussed.

S 1418 SEX AND TRANSPORTERS IN THE GI TRACT AND KIDNEY.


This presentation examines sex-related differences in the kinetics of toxic metals in the GI tract and kidney. Cation transporters play a major role in essential and toxic metal uptake from water and diet. Underlying nutritional status, such as zinc, iron, and calcium, are altered by gender and ultimately influence transporter expression. Nutritional deficiencies result in gender specific up-regulation of transporters, thereby facilitating the transport of competing toxic metals such as lead and cadmium. The latest findings on the role of gender on regulation of transporter genes in vivo will be discussed.

S 1419 SEX DIFFERENCES, CIGARETTE SMOKE, AND INFLAMMATORY HEART DISEASE: ROLE OF ALTERNATIVELY ACTIVATED MACROPHAGES.

D. Fairweather1,2 and M. I. Coronado1. (1) Environmental Health Sciences, Johns Hopkins University, Baltimore, MD and (2) Pathology, Johns Hopkins University, Baltimore, MD.

Recent clinical studies indicate the importance of sex differences in the pathogenesis of heart disease with men at a higher risk of developing heart disease than women. This presentation will focus on the interplay between sex, infectious disease, inflammation and cigarette smoke exposure. Male BALB/c mice develop significantly more severe coxsackievirus B3 (CVB3)-induced myocarditis and dilated cardiomyopathy (DCM) than females. Males have significantly more inflammation in the heart than females with more CD11b+ cells including TL1R4+ macrophages (Mac) and a predominating Th1-type immune response with significantly higher proinflammatory cytokines including IL-1β, IL-18 and IFN-γ. Microarray analysis compared males and females with CVB3 myocarditis revealed that approximately 500 genes were significantly upregulated in males during acute myocarditis, falling into three categories: biomarkers of heart disease, markers for CD11b+ Mac and anti-oxidant response genes. Males also have more TL1R4+IL-1β alternatively activated Mac (M2) than females, which contribute to fibrosis and DCM. After exposure to cigarette smoke (CS), we found that pericarditis and DCM were significantly increased but myocarditis was decreased in males. We hypothesize that CS exposure skews the immune response to increase M2 resulting in less acute inflammation in the heart but increased chronic DCM.

S 1420 MECHANISMS OF GENDER DIFFERENCES IN CHEMOTHERAPY INDUCED CARDIAC TOXICITY.

K. Gabrielson. Molecular and Comparative Pathobiology, The Johns Hopkins University School of Medicine, Baltimore, MD.

More patients with malignant diseases are being cured to survive for longer periods, yet the survival rates are much better for females than males. This presentation will focus on studies that demonstrate gender differences in gene expression and signal transduction pathways controlling mitochondrial oxidative stress, hypertrophy and protein translation after chemotherapy. For example, genes related to protein synthesis (translation initiation factors and ribosomal subunits) are up-regulated in DOX treated females compared to DOX males. Secondly, uncoupling proteins like UCP2, found within the inner mitochondrial membrane, reduce superoxide production and are up-regulated in DOX females. Lastly, estrogen regulates the expression and activation of the protective protein eNOS (endothelial Nitric Oxide Synthase -NOS3) through the AKT pathway and our recent findings demonstrate that eNOS expression is significantly higher in doxorubicin treated females. Differential expression of AKT pathway in the heart during doxorubicin toxicity will also be discussed. In summary, this presentation will focus on programmatic signals transcription and mitochondria biology, both affected by gender specific gene expression.

S 1421 MITOCHONDRIAL TOXICITY IN DISEASE AND DEATH.

B. Zhivotovsky. Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Stockholm, Sweden.

Mitochondria play a central role in cell life and death and are known to be important in a wide range of diseases. Many attempts were undertaken to develop drugs that target mitochondria and suggested to be used to treat mitochondrial dysfunctions associated with various diseases. However, it is known that such drugs induce mitochondrial toxicity. At the same time mitochondria are central to many chronic toxicities the details of the mechanisms remain unknown and effective preventive strategies have not been established. Therefore our approach to explore these issues will be to delineate therapy-related toxicities, which are essential to understanding the mechanisms behind the role of mitochondria in disease and death. To achieve this goal, an interaction between both fundamental and applied research is important. Our panel of experts represent different areas of toxicology, from academia to industry, which will provide attendees a varying perspectives on this important issue which will lend itself to broad and deep discussions relative to where the field of toxicology is headed.

S 1422 MITOCHONDRIA AS TARGET FOR CHEMOTHERAPY.

B. Zhivotovsky, V. Gogpoydaze, E. Norberg and S. Orenius, Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Stockholm, Sweden.

Disturbance of mitochondrial vital functions, e.g. production of ATP, calcium buffering capacity, and generation of reactive oxygen species, can be potentially involved in disease pathogenesis. Neurological disorders caused by mitochondrial deterioration are often associated with cell loss within specific brain regions. In contrast, in tumor cells the glycolytic switch (the "Warburg effect") and accompanying suppression of mitochondrial activity decreases cell death potential of these organelles and causes resistance of tumors to chemotherapy. Therefore, the unusual behavior of mitochondria and their involvement in the initiation and/or regulation of cell death pathways make targeting of these organelles a promising strategy for tumor cells elimination. Indeed, a redox-silent analogue of vitamin E, α-tocopheryl succinate (VTS) causes apoptotic cell death through targeting mitochondria of various tumor cells. In particular, α-TOS stimulates production of reactive oxygen species, induces mild uncoupling of mitochondria, and compromises mitochondrial Ca2+ buffering capacity. In addition, α-TOS generates cytotoxic Ca2+ transients with subsequent accumulation of these ions in mitochondria, a prerequisite step for induction of mitochondrial permeability transition. Ca2+-induced mitochondrial destabilization co-operates with Bax-mediated outer mitochondrial membrane permeabilization and cytochrome c release. Prevention of mitochondrial Ca2+ accumulation significantly mitigates apoptotic response. Accumulating evidence suggest that targeting of mitochondria with modulators of cellular bioenergetics can be an important step in the sensitization of tumors for the combined chemo- and radiotherapy.

S 1423 MITOCHONDRIAL REDOX PROTEOME: SUSCEPTIBLE SITE OF CHRONIC TOXICITY.

D. P. Jones, Y. Go and J. Pohl. Division of Pulmonary Medicine, Emory University, Atlanta, GA.

Mitochondrial oxidative stress results in both macromolecular damage and disruption of redox control mechanisms. While the former is critically important in mitochondrial DNA damage, the latter occurs through effects on the mitochondrial redox proteome. Mitochondrial redox signaling and control pathways differ from the high-flux electron transfer pathways for ATP production in that they are low-flux pathways which use cysteine residues of proteins as thiol switches for metabolic

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regulation. These switches are oxidized by endogenously produced H2O2 and reduced by thioredoxin-2 (Trx2) and mitochondrial GSH systems, and are central components of the mitochondrial redox proteome. These switches are important sites of vulnerability to environmental toxicants, including oxidants, electrophiles and heavy metals. New methods to map the redox dependence of specific mitochondrial proteins include redox-western blotting and mass spectrometry-based redox proteomics. Studies of Trx2 show that the protein is modified by reactive electrophiles and sensitive to oxidation by trace metals, mitochondrial respiratory inhibitors and deficiency in mitochondrial H2O2 production than wt littermates, suggesting that a fraction of mitochondrial protein thiols is dynamically regulated. The emerging data from studies of the mitochondrial proteome show that environmental exposures, age, diet and availability of metabolic fuels, interact to determine the oxidation of Trx2. Kinetic limitation of electron flow from Trx2 to peroxiredoxin-3 indicates that these changes affecting Trx2 redox state affect a diverse subset of mitochondrial proteins which includes key sites of susceptibility to chronic toxicity.

1424 IRON, LYSOSOMAL FRAGILITY, AND MITOCHONDRIAL DYSFUNCTION.

J. J. Lemasters, Center for Cell Death, Injury and Regeneration, Departments of Pharmaceutical & Biomedical Sciences and Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC.

Iron exacerbates many toxicities and disease processes in liver and other organs and is a catalyst for hydroxyl radical formation from superseroxide and hydrogen peroxide. Lysosomes are a cellular repository of chelatable iron. Various injuries and stresses lead to lysosomal breakdown and release of chelatable iron, which is taken up into mitochondria by the mitochondrial calcium uniporter to promote intramitochondrial generation of hydroxyl radicals and reactive oxygen species (ROS). ROS formation promotes onset of the mitochondrial permeability transition and both apoptotic and necrotic cell death. Desferal, an iron chelator, and inhibitors of the calcium uniporter prevent mitochondrial iron uptake, ROS formation and consequent cell death in oxidative stress and hyposxia/ischemia. This pathway of intracellular iron translocation is a potential therapeutic target against oxidative stress-mediated toxicity. The common occurrence of iron imbalance in aging and the complex interactions of toxic metals, oxidative stress and mitochondria-mediated toxicity make this a critical subject for future human toxicology research.

1425 MITOCHONDRIAL OXIDATIVE STRESS: IMPLICATIONS FOR CELL DEATH.

S. Ottenius, E. Norberg, V. Gogadze and B. Zhivotovsky, Institute of Environmental Medicine, Division of Toxicology, Karolinska Institute, Stockholm, Sweden.

In addition to the long established role of the mitochondria in energy metabolism, regulation of cell death has emerged as a second major function of these organelles. This, in turn, seems to be intimately linked to their role as the major intracellular source of reactive oxygen species (ROS), which are mainly generated at Complex I and III of the respiratory chain. Excessive ROS production can lead to the oxidation of macromolecules and has been implicated in mtDNA mutations, aging, and cell death. Although mitochondrial dysfunction per se can result in ATP depletion and necrosis, these organelles are also involved in the regulation of apoptotic cell death by mechanisms that have been conserved through evolution. Hence, many toxic agents target the mitochondria and cause the release into the cytosol of cytochrome c and other pro-apoptotic proteins, which can trigger caspase activation and other key events in apoptosis. Cytochrome c release occurs by a two-step process that is initiated by the dissociation of the hemoprotein from its binding to cardiolipin in the inner mitochondrial membrane (IMM). Similarly, Apoptosis Inducing Factor (AIF) must also be detached from the IMM, before it can be exported from the mitochondria into the nucleus. This occurs by proteolytic cleavage of the peptide chain that anchors it to the IMM. AIF cleavage is catalyzed by mitochondrial calpain and preceded by carbonylation of AIF triggered by mitochondrial ROS production. Subsequent release of cytochrome c and AIF into the cytosol occurs via pores in the outer mitochondrial membrane formed by pro-apoptotic Bcl-2 family proteins, or by Ca2+/ROS-induced mitochondrial permeability transition, although the latter mechanism might be more closely associated with necrotic cell death. Hence, it is apparent that mitochondrial ROS generation is critically involved in the control of both apoptotic and necrotic cell death and an important mediator of mitochondrial toxicity.

1426 METHODS TO DETECT MITOCHONDRIAL TOXICITY CAUSED BY ANTI-RETROVIRAL AND ANTIBACTERIAL THERAPY.

S. Nadanaciva, Pfizer R&D, Groton, CT. Sponsor: B. Zhivotovsky.

Nucleoside reverse transcriptase inhibitors (NRTIs) given in the treatment of HIV infection cause mitochondrial toxicity since DNA polymerase gamma, the enzyme responsible for replicating mtDNA, is highly sensitive to certain NRTIs. Many of these drugs have received Black Box warnings by the Food and Drug Administration. Another class of drugs, antibacterials which are bacterial protein synthesis inhibitors, can also cause mitochondrial toxicity since there is a structural similarity between bacterial ribosomes and the host’s mitochondrial ribosomes. Both classes of compounds have the overall effect of reducing the level of proteins encoded by mtDNA. In order to reduce compound attrition caused by these compounds, methods to detect changes in mtDNA-encoded protein levels have been developed. This talk will focus on two of these methods. One of these is a high content imaging assay and the other is a lateral-flow immunoassay. The levels of a mtDNA-encoded protein made on mitochondrial ribosomes and a nuclear DNA-encoded protein level made on cytosolic ribosomes are measured in both assays. The results from these assays show that (1) the anti-retrovirals impair mtDNA-encoded protein levels with rank order of potency: 2’-3’-dideoxyxycytidine > 2’-3’-dideoxyinosine > Stavudine > Lamivudine, Abacavir; (2) some of the antibacterials which inhibit either bacterial protein synthesis (e.g. the oxazolidinones) or bacterial DNA synthesis (e.g. the fluoroquinolones) impair mtDNA-encoded protein levels; in contrast, antibacterials which inhibit bacterial cell wall synthesis or disrupt the bacterial lipid membrane do not reduce mtDNA-encoded protein levels. The utility of (a) the high content imaging assay as part of a suite of screens positioned early in the drug discovery process for identifying mitochondrial toxicity and (b) the lateral-flow immunoassay for analyzing clinical samples of patients on anti-retroviral therapy will be discussed.

1427 THE FETAL BASIS OF ADULT DISEASE.

D. A. Delker1 and E. J. Tokar.1 1NCI at NIEHS, Research Triangle Park, NC and 2University of Utah School of Medicine, Salt Lake City, UT.

Recent studies provide convincing evidence for the fetal basis of adult disease. Gestation is a period of high sensitivity to toxicants, with a variety of maternal exposures leading to consequent diseases such as cancer, atherosclerosis, hypertension, obesity, and diabetes in the offspring often much later in adulthood. There is strong suspicion that embryonic/fetal stem cells (SCs) are key targets in the transplacental chemical attack that is the etiological basis of these diseases, in part because of their relative abundance and their role in organogenesis and differentiation. The long latency period between in utero exposure and development of adulthood diseases is consistent with lesions in conditionally immortal SC populations with their limitless capacity for self-renewal. Beginning with the theory of the fetal basis of adult disease (the Barker Hypothesis) and how it relates to alterations in SCs and SC numbers, the symposium will then describe the impact of transplacental arsenic exposure on skin SC dynamics, illustrating how early life arsenic exposure plays a role in skin cancer much later in life. The transplacental arsenic-induced changes in liver programming associated with accelerated atherosclerosis in adulthood, and the effects of maternal lead (Pb) exposure on the hypothalamic-pituitary-adrenal axis and development of Pb-associated adult diseases will then be covered. Next, in vitro SC model systems demonstrating arsenic transforms SCs into a pluripotent cancer SC (CSC) phenotype and how this phenomenon may be specific to arsenic as opposed to other carcinogenic metals (e.g. cadmium) are described. Concluding this session, our panel of experts will discuss genomic profiling of SC signaling pathways in adult animals following environmental chemical exposure and how the activation of these pathways in vivo may predict tumor outcome. This session will be of interest to those researching development exposure to metals and other toxicants, molecular toxicology, molecular mechanisms involved the regulation of SCs, and the initiation of CSCs, as well as those interested in the fetal basis of adult disease.

1428 MODULATION OF HUMAN STEM CELLS DURING IN UTERO EXPOSURES TO TOXICANTS: A MECHANISTIC EXPLANATION TO THE BARKER HYPOTHESIS.

J. E. Trusk. Department Pediatrics/Human Development, Michigan State University, East Lansing, MI.

In principal, chemicals might affect cells by mutagenesis, cell killing or alteration of gene expression (epigenetic toxicity). It is assumed that chemical toxins/ toxicants are not mutagenic to genomic DNA of the stem cells; however at non-cytotoxic lev-
els, they disrupt homeostatic control of proliferation, differentiation, senescence and apoptosis by triggering oxidative stress-induced intra-cellular signaling in adult stem cells, progenitor and differentiated cells, and in between tissues, to modulate gene expression and gap junctional intercellular communication (GJIC). Because adult stem cells and GJIC exist in all organs, disruption of the quality and quantity of stem cells and gene expression, especially during embryogenesis and fetal development, can lead to both (a) immediate birth defects and (b) chronic diseases, such as cancer, atherosclerosis, diabetes, immunotoxicity, reproductive- and neurologi- cal- disorders later in life. The Barker hypothesis might be explained by pre-natal exposures that lead to altered stem cell numbers, thereby increasing or decreasing the risk to diseases, dependent on stem cells, later in life. Non-mutagenic chemicals, which can induce differentiation and GJIC of stem cells, can be either beneficial or detrimental.

**1429 FETAL ARSENIC EXPOSURE ENHANCES SKIN CANCER IN ADULTHOOD WITH CONTEMPORANEOUS DISTORTION OF TUMOR STEM CELL DYNAMICS.**

M. P. Waalkes, NCI at NIEHS, Research Triangle Park, NC.

Arsenic is a carcinogen with transplacental activity that appears to impact human skin stem cell population dynamics in utero. Keratinocyte stem cells (KSCs) are thought to be an important target in skin cancer carcinogenesis. Thus, we investigated the effects of in utero arsenic exposure on skin cancer in Tg.AC transgenic mice, a strain sensitive to skin carcinogenesis via activation of the v-Ha-ras transgene likely in KSCs. After in utero arsenic exposure, offspring received topical 12-O-tetradecanoyl phor- bol-13-acetate (TPA) throughout adulthood. Arsenic alone had no effect, while TPA alone induced papillomas and squamous cell carcinomas (SCC). With in utero ar- senic exposure before TPA mice developed well over twice as many SCC than with TPA only. Tumor levels of v-Ha-ras transcript were three times higher with arsenic plus TPA than with TPA alone, and v-Ha-ras was over expressed as an early event in arsenic treated fetal skin. Tumor transcript levels of CD34, a KSC marker, and Rae1, a key gene in KSC self renewal, were greatly increased with arsenic plus TPA versus TPA alone, and were similarly elevated in arsenic treated fetal skin. Over expression of Rae1 protein and greatly increased CD34 positive putative KSCs were observed in tumors induced by arsenic plus TPA. Thus, in utero arsenic exposure, though alone oncogenically inactive, stimulates skin cancer in association with distorted skin stem cell signaling and population dynamics, implicating stem cells in the fetal basis of cancer in adulthood.

**1430 TRANSPLACENTAL ARSENIC EXPOSURE INDUCED CHANGES IN LIVER PROGRAMMING ASSOCIATED WITH ACCELERATED ATHEROSCLEROSIS.**

J. C. Stats1, A. Singh2, T. Knudsen3, E. Rouchka4, M. S. Ko5, Y. Piao5, N. O. Ngalame6, J. Arteel7, G. Arteel7 and S. Srivastava8.1 Pharmacology & Toxicology, University of Louisville, Louisville, KY; 2Molecular Cellular & Comparative Biology, University of Louisville, Louisville, KY; 3Computer Engineering & Computer Science, University of Louisville, Louisville, KY; 4Medicine, University of Louisville, Louisville, KY and 5Laboratory of Genetics, National Institute on Aging, Baltimore, MD.

In utero exposure to toxicants can predispose to development of chronic adult dis- eases. Cardiovascular disease is elevated in areas of endemic exposure to arsenic in drinking water. The role that in utero arsenic exposure plays in development of ath- erosclerosis underlying the cardiovascular disease is unknown. We showed that in utero arsenic exposure accelerates atherosclerosis in the ApoE-knockout mouse. Liver damage plays a central role in development of the inter-related chronic diseases metabolic syndrome, diabetes and atherosclerosis. Using a genomics ap- proach, we investigated effects of in utero arsenic exposure on liver developmental programming. Pregnant dams were provided drinking water with or without 85 mg/L NaAsO2 from gestational day 8 – 20. Abundances of both mRNAs and miRNAs were evaluated by microarray analyses of total RNA from livers of exposed and unexposed progeny on the day of birth (PND1) and at age 10 weeks (PND70). Plasma biomarkers of liver injury were elevated in 10 week old mice exposed to ar- senic in utero. These results along with analyses of the microarray data are consist- ent with epigenetic changes altering liver development and hepatic inflammatory responses that may contribute to the acceleration of atherosclerosis caused by prena- tal arsenic exposure in ApoE-knockout mice. Supported by PHS grants R21ES015812, R01ES011314 & P30ES014443, UofL Center for Genetics and Molecular Medicine pilot grant, UofL Collaborative Planning and Development Grant and Intramural Research Program of NIA/NIH.

**1431 PERMANENT EFFECTS OF MATERNAL LEAD (PB) EXPOSURE ON THE HPA AXIS: A BIOLOGICAL UNIFYING MECHANISM FOR PB-ASSOCIATED ADULT DISEASES.**

D. A. Cory-Slechta, Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY.

A major hypothesis underlying early life permanent physiological programming in- volves fetal exposure to excess glucocorticoids, leading to increased risk of cardio- vascular (hypertension), metabolic (obesity), neuroendocrine (type 2 diabetes) and various behavioral and psychiatric disorders (schizophrenia, attention deficit disor- der). Our studies demonstrate that maternal only Pb exposure may produce similar permanent physiological changes through effects on the hypothalamic-pituitary-ad- renal (HPA) axes. Specifically, developmental only exposures to low levels of Pb in rats initiated 2 mos prior to breeding of dams and continuing until offspring were weaned and associated with blood Pb values of approximately 10-35 ug/dL, produce permanent HPA axis dysfunction in offspring of both genders. These changes were reflected in dynamic alterations in corticosterone levels throughout adulthood, al- tered behavioral and corticosterone responses to stress challenges, and reductions in the ability of dexamethasone to suppress corticosterone. The latter suggest alter- ations in delayed glucocorticoid negative feedback, and resulted in a hypercorti- solism which was more prominent at the lower than higher blood Pb levels. Consistently, these findings provide a unifying mechanism for the diverse diseases and disorders that have now been associated with environ- mental Pb exposure, including obesity, hypertension, diabetes, anxiety, schizophrenia and depression, all of which have been related to HPA axis dysfunction. In ad- dition, elevated glucocorticoids lead to hippocampal neuronal death and have been postulated to play a role in Alzheimer’s disease, which has also recently been linked to environmental Pb exposure. Studies are needed to define the lowest Pb exposure levels associated with such effects and their possible epigenetic basis. Moreover, be- cause these effects are induced by maternal Pb exposure, they underscore the need for blood Pb testing in pregnant women at risk of elevated Pb exposure.

**1432 ARSENIC-INDUCED STEM CELL INITIATION PRODUCES A CANCER STEM CELL PHENOTYPE DURING MALIGNANT TRANSFORMATION.**

E. J. Tokar, NCI at NIEHS, Research Triangle Park, NC.

Emerging evidence indicates that cancer stem cells are the true malignant cells within tumors and are carcinogenically initiated from normal stem cells or their close, partially differentiated progeny. Recent reports show that arsenic has transplacental carcinogenic activity in mice and probably humans. In mice, fetal ar- senic exposure causes tumors and oncogenic lesions throughout the urogenital sys- tem much later in life. This greatly delayed response suggests that arsenic targets a stem cell population, possibly producing quiescent cancer stem cells that then in- duce cancer upon stimulation much later in life. This presentation will first discuss arsenic-induced transformation of human and rodent stem/progenitor cells, fol- lowed by a description of primary events and genetic alterations involved in this ini- tiation of cancer stem cells. These alterations include an early loss of stem cell self- renewal gene expression (p63, ABCG2, BMP-1, SHH, OCT-4, NOTCH-1) that is subsequently reversed as the tumor suppressor gene PTEN is progressively sup- pressed and cancer stem cell phenotype acquired. This phenotype appears to be as- sociated with aberrant expression of at least some imprinted genes. This indicates that arsenic, a ubiquitous environmental contaminant and known human carcino- gen, can directly transform stem/progenitor cells into a pluripotent cancer stem cell phenotype. A comparison of acquired cancer stem cell charactersitics in isogenic ar- senic-, cadmium, and N- methyl-N-nitrosourea-transformed cell lines indicates that arsenic is much more prone to modifying stem cell populations and precartiopating cancer stem cell formation. Thus, arsenic-induced carcinogenic initiation appears to be at the stem cell level.

**1433 EPIGENETIC SIGNALING AS A TARGET FOR CHEMICAL TOXICITY AND CANCERGENESIS.**

D. A. Deklerk1 and W. O. Ward2. 1University of Utah School of Medicine, Salt Lake City, UT and 2U.S. Environmental Protection Agency, Research Triangle Park, NC.

Epigenetic regulation of cell differentiation and organ development is a natural process targeted in disease and chemical toxicity. Stem cells and their progenitors are essential for the development and maintenance of normal tissue function. These cells are characterized by specific gene methylation patterns and RNA profiles in- cluding microRNAs and alternative RNA splicing not observed in differentiated cells. Recent studies suggest that the disruption of epigenetic signaling occurs very early in the cancer process and that epigenetic abnormalities are more widespread.
than genetic mutations which have long been the hallmark of cancer. Since cancer is considered a disease of clonally expanded de differentiated cells with stem cell-like properties, the genomic analysis of signaling pathways involved in stem cell maintenance and renewal in vivo might provide a useful alternative for estimating cancer risk following short term environmental chemical exposure. We have identified alterations in the Wnt signaling pathway in target tissues as early as two weeks following chemical carcinogen exposure while little or no change was observed in this pathway following non-carcinogen exposure. The observed alterations in Wnt signaling gene transcripts were also dose-dependent and correlated well with the relative potency of the chemical carcinogen. Bioinformatic analysis of public gene expression data from cancer studies reveals clustering of Wnt signaling genes into receptor/ligand and second messenger gene families during stem cell growth. In this talk research that facilitates the genomic characterization of alterations in these signaling pathways as predictors of the carcinogenic potential of environmental chemicals.

1434 CURRENT THINKING AND EXPERIENCES ON DEVELOPMENTAL AND REPRODUCTIVE SAFETY ASSESSMENT OF BIOTHERAPEUTICS.

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As scientific thinking and regulatory expectations around highly target-specific biotherapeutics have evolved, it has become increasingly difficult to design meaningful nonclinical strategies that reduce uncertainty around the risk of effects on human reproduction and development. Importantly, these nonclinical studies are likely the most reliable method available to prevent drug-induced birth defects and infertility since clinical evaluation of these endpoints is unethical or rare. These studies should generally be in compliance with ICH S5, which is designed primarily to detect toxicity to reproduction and development (hazard identification). From ICH S5 relatively standard nonclinical strategies for small molecules have evolved, but for practical, technical, and sometimes ethical reasons may have limited value for large molecules or vaccines (issues ranging from placental transfer to limited off-target toxicity). Although most biological effects of biotherapeutics have an origin in modification of a target or target signaling, it is uncommon to have unexpected effects on reproduction and/or development since regulation/function of the target during these lifestages is often not well understood, particularly for novel drug targets. As described in ICH S6, for biotherapeutics careful scrutiny of the nonclinical strategy and conduct of specific studies is necessary to appropriately account for many issues, particularly species specificity, immunogenicity, biological activity and/or elimination half-life. In order to adhere to ever-changing regulatory expectations, minimize the use of animals; and improve the performance of safety assessment/toxicology around potential treatment-related effects on reproduction and development; innovative strategies using a combination of animal models (e.g., transgenic) and study designs (e.g., use of homologues or combined pre/postnatal development in nonhuman primate) are currently being developed and applied by many companies.

1435 CURRENT REGULATORY EXPERIENCE AND PROPOSED MODIFICATIONS TO ICH S6.


The regulatory approach in the preclinical assessment of new biotechnology-derived human pharmaceuticals in the ICH S6 Guideline is explicitly different from the common approach for conventional small molecule, chemically-synthesized pharmaceuticals. The development of a large number of monoclonal antibodies for a broad population including women of child-bearing potential revealed that the minimal attention to this group of products in the present S6 Guideline merits an update in the description of the regulatory approach. In preparing the revision of the guideline we have evaluated the experience with respect to developmental and reproductive toxicity (DART) testing for the products with a European marketing authorization, with a further emphasis on the monoclonal antibodies and related products. This evaluation has revealed that for protein hormones and other proteins the classical DART approach of two species (mainly rats and rabbits) has been used to test the reproductive safety. For 15/20 approved monoclonal antibodies DART has been tested. The Cynomolgus monkey showed to be the species used in the majority of the cases (10 out of 15). The human fetal risk of the exposure to monoclonal antibodies is more associated with functional effects derived from influencing development in the latter part in pregnancy rather than with malformations resulting from interference with organogenesis in the first trimester (due to limited fetal exposure during this developmental window). For compounds which are pharmacologically active in non-human primates only a DART study in a single, i.e. this species is sufficient. Fertility assessment may be covered in the repeated dose toxicity test if functional assessment is not feasible (e.g. monkey). The revision of the S6 will focus on the redundancy of a classical embryo-fetal developmental toxicity study for monoclonal antibodies recommending a rather integral approach e.g. with an enhanced peri-postnatal development study covering nearly the complete period of gestation.

1436 PRECLINICAL STRATEGY CONSIDERATIONS FOR ASSESSING THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY POTENTIAL OF BIOPHARMACEUTICALS.

J. Cavagnero. Access BIO, Boyce, VA.

The “principles” of DART testing for biopharmaceuticals are similar to those for small molecule pharmaceuticals and in general follow the guidance outlined in ICH S5 (R2). However, because many biopharmaceuticals are species-specific, alternate approaches or “practices” are generally needed to evaluate DART potential as outlined in the initial ICH S6 guidance. The need for this “case-by-case approach” is dictated by differences in product attributes. This presentation provides both a framework for developing DART testing strategies for biopharmaceuticals, in the context of the overall clinical development strategy, as well as an overview of the state of DART testing of biopharmaceuticals. The various strategies that have been successfully implemented over the past two decades will be summarized based upon regulatory reviews and the recently published BioSafe White Paper (Birth Defects Res (Part B), 33:176-203, 2009). Lessons learned will be applied to future strategies in the context of novel compounds now entering development. Current challenges will be highlighted as well as the importance of communicating and managing potential DART risks of biopharmaceuticals.

1437 CHALLENGES AND SOLUTIONS FOR EVALUATING THE DEVELOPMENTAL TOXICITY POTENTIAL OF BIOTHERAPEUTICS.

L. Andrews. Genzyme Corporation, Framingham, MA.

As the development of biotherapeutics becomes a more advanced science based challenge, the selection of relevant animal models, utility of traditional species and alternatives to traditional safety approaches are becoming more accepted and in fact, necessary. The challenges of biotherapeutics become especially evident when considering the most appropriate science based approach for the conduct of developmental and reproductive studies. Designing an informative developmental and reproductive toxicity study for a biotherapeutic can be a challenge in light of the need to utilize alternative approaches that may be divergent from a more traditional Segment I, II, III approach and include utilization of two species. Alternatives to the traditional safety approach include the use of homologous proteins, transgenic animals, animal models of disease as well as state of the art non-invasive, non-terminal technologies such as high resolution imaging and scanning methods. In addition, a science based approach to rationale study design has allowed for a better use of animals through the development process. Study design considerations must be addressed in order to most effectively utilize animals and wherever possible reduce the need for large numbers and multiple studies. The opportunities and challenges for these approaches with respect to developmental and reproductive studies as well as the approach to implementing these areas to help reduce animal use and advance the science of biotechnology drugs will be discussed. A specific example of a monoclonal antibody in development will be illustrated including a discussion of regulatory feedback and alternatives to a traditional approach.

1438 CASE STUDIES: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STRATEGIES EMPLOYED TO SUPPORT THE REGISTRATION OF GOLIMUMAB AND USTEKINUMAB.


Golimumab and ustekinumab are fully human monoclonal antibodies to soluble cytokines (tumor necrosis factor α and IL-12/23, respectively). Cynomolgus monkeys were identified as biologically relevant species for toxicity studies for both antibodies. Common goals of the developmental toxicity studies for both antibodies
to characterize exposure of the conceptus and identify potential adverse effects on the developing immune system by incorporating morphological and/or functional evaluations of the immune system. For golimumab this was accomplished with two studies in monkeys: an embryofetal development study (with dosing from gestation days (GD) 20-50) and a pre and postnatal (PPD) development study (with dosing from GD 50 to lactation day (LD) 33). For ustekinumab two EFD studies and a combined EFD/PPD study (with dosing from GD20 to LD 33) were performed in monkeys. Maternal and/or fetal hormone measurements were included in the ustekinumab studies. A male fertility study with ustekinumab was conducted in monkeys but a supportive female fertility study with a surrogate anti-mouse IL-12/23 antibody was performed. A combined male/female fertility study design was supportive of golimumab registration. Although both programs targeted soluble cytokines regulating immune responses, different DART strategies were needed based on the nature of similar products and pathway-specific findings in peer reviewed clinical and non-clinical publications. A comparison of the DART programs for golimumab and ustekinumab illustrates the role of a flexible, science-based, case-by-case approach to evaluate developmental and reproductive toxicity for biopharmaceuticals.

W 1439 STATE OF THE SCIENCE ON REPRODUCTIVE AND DEVELOPMENTAL SAFETY ASSESSMENT ON VACCINES AND ADJUVANTS.
S. J. Gould, Sanofi Pasteur, Lyon, Marcq L’Etanol, France.

To support the vaccination of women of child bearing potential, it is necessary to examine the potential harmful effects of a vaccine on the development and growth of the embryo and fetus following exposure of the female to the vaccine from implantation through to the end of pregnancy, with follow-up of the offspring through weaning (DART study). The FDA guidance document on reproductive and developmental safety evaluation of preventative and therapeutic vaccines for infectious diseases provides a good outline on the principles of testing. However, it may not address all potential issues. Vaccines are diverse product, which may be live attenuated, inactivated or fractions of microorganisms, polysaccharides, toxoids, recombinant proteins/polypeptides, DNA or vector based, and may be administered with or without an adjuvant. As such, the design of a developmental study should be case by case and designed to detect and characterize vaccine specific adverse events. For a vaccine, in general, only one species is necessary: the species selected should develop an immune response; the fetus should be exposed to maternal antibodies (Ab), and should also be amenable to fetal and post natal examination. For an adjuvant, two species may be necessary. There are some elements that require further consideration, which may not have been addressed specifically in the guidelines. For example: the organs essential to of the immune system are not typically assessed in DART studies, but should this be revisited? Live attenuated vaccines or novel vector vaccines, may induce viremia, which is considered as part of the general vaccine safety evaluation, but is not considered in the DART guidelines. What about adjuvants? Especially those defined more as a chemical entity than a biological, what is the most appropriate species, study design etc. This presentation will consider the rationale for designing and conducting developmental studies, and will consider some of the unmet needs and challenges. The talk will be supported where possible by proven non clinical strategies from vaccines either licensed or currently in development.

W 1440 NOVEL RESEARCH APPROACHES, ANIMAL MODELS, AND CLINICAL EXAMPLES IN TRANSLATIONAL TOXICOLOGY.
S. K. Ramaiah, Drug Safety Research and Development, Pfizer Global Research and Development, Saint Louis, MO.

Translational toxicology is defined by the ability to translate preclinical animal safety findings to human to successful drug development. Preclinical safety studies are mostly carried out in both rodent and a non-rodent species, with the primary goal to demonstrate or identify target organ effects translatable to human health. In addition these studies also enable selection of the dose for first-in-human studies, demonstrate a margin of safety between the efficacious and toxic doses, and establish mechanisms for monitoring safety during clinical trials such as biomarkers. In order to demonstrate translatability, it is critical to develop the most relevant animal model and to select the appropriate endpoints that can accurately predict human toxicity. If a safety issue is identified, it is of utmost importance to have the most sensitive and specific translatable biomarker of organ toxicity in addition to the ideal assay platform to monitor such biomarkers clinically. Without these, the incidence of drug failure due to toxicity during clinical development and the occurrence of morbidity and mortality in human patients will continue to increase. The goal of this session will be to provide novel research approaches and examples to address certain gaps in drug development to ensure clinical translatability. The application of novel research approaches, animal models, and biomarker platforms will be discussed from clinical and nonclinical scientists actively engaged in these areas.

W 1441 WHY DO ANIMAL MODELS FAIL TO PREDICT IDIOSYNCRATIC HEPATOTOXICITY IN HUMANS.
A. Repay, Global Patient Safety, Eli Lilly, Indianapolis, IN.

Idiosyncratic drug induced liver injury (DILI) is an uncommon and currently unpredictable drug related adverse event. Despite increasing efforts to understand and predict this group of adverse events, idiosyncratic DILI remains a major concern for drug development and patient safety, and continues to be the leading cause for regulatory action. Preclinical animal studies are still largely unsuccessful in predicting idiosyncratic DILI. Furthermore, to date there is no universally accepted animal model for the investigation or prediction of this adverse effect. Since clinical trials typically do not include enough subjects to detect idiosyncratic DILI, this type of liver injury is detected only post marketing when hundreds of thousands or millions of patients are exposed to the drug. The risk of developing idiosyncratic DILI likely involves a complex interaction between the chemical properties of the drug, genetic factors and environmental factors. Reproducing these risk factors in animals has proven to be a challenging and commonly unattainable task. Recent examples will be presented to highlight the current thinking on this topic and illustrate some of the difficulties in the use of animal models for understanding and predicting idiosyncratic DILI.

W 1442 NOVEL TRANSLATIONAL RESEARCH APPROACHES TO UNDERSTAND AND PREDICT DRUG INDUCED LIVER INJURY.
P. B. Watkins, Center for Drug Safety Sciences, University of North Carolina, Chapel Hill, NC.

The type of DILI that is most problematic is “idiosyncratic” meaning that only a very small fraction of treated patients are susceptible to the DILI. Current preclinical models, even “humanized” ones, do not reliably identify molecules that are safe for the liver and, conversely predict liabilities in molecule that are in fact safe for the liver. Reliable preclinical testing will probably not be developed until there is greater understanding of the mechanisms underlying idiosyncratic DILI. The Hamner UNC Center for Drug Safety Sciences has been building novel research programs that are designed to bridge the gap in knowledge between preclinical models and patients. Research programs to be discussed include using panels of inbred mouse strains to identify specific genes and pathways that underlie DILI susceptibility and that can be used to generate hypotheses that are testable in the human DILI gene banks assembled by the Severe Adverse Events Consortium (SAEC) and the Drug Induced Liver Injury Network (DILIN) (A. Harrill et al, Genome Research, in press). A second research project is exploring the observation that liver derived mRNAs are present in circulating plasma during DILI and may be allow “real time” toxicogenomic studies of human liver. Finally, the center is collaborating with Extelos and the FDA to develop the DILI Physiklab platform. This computer-based platform consists of simulations of interacting pathways that underlie susceptibility to DILI. When completed, it will improve understanding of many aspects of DILI, including species differences in DILL susceptibility.

W 1443 KIDNEY AND VASCULAR TOXICITIES OF VEGF SIGNALING PATHWAY INHIBITORS: MECHANISM-DEPENDENT BIOMARKERS FOR TREATMENT EFFICACY?
B. D. Humphreys, Translational Research in Kidney Repair, Harvard University, Boston, MA. Sponsor: S. Ramaiah.

VEGF signaling pathway (VSP) inhibitors are a promising and rapidly growing new anti-angiogenic chemotherapy class that block tumor angiogenesis through inhibition of endothelial cell VEGF receptor signaling. It had been presumed that
ing that knowledge into AOP models. Finally, because species extrapolation is a central challenge in ecological risk assessment, the workshop examined how to determine conservation of AOPs among species and use this information in predicting species sensitivity to support ecological risk assessments. This session will summarize the results of the SETAC effort and invite discussion with SOT members regarding development of an integrated toxicity testing paradigm that supports both human health and ecological risk assessment.

1444 LEAD OPTIMIZATION STRATEGIES AND NOVEL BIOMARKER TECHNOLOGIES TO ENSURE DRUG SAFETY.

J. S. Oxer, Pharmacokinetica, Dynamics and Metabolism, Pfizer, Chesterfield, MO. Sponsor: S. Ramaih.

Because of the increased awareness and regulatory emphasis on drug safety issues during clinical trials and product post-marketing use, there is a need for newer biomarker technologies and approaches to better quantify and validate the most sensitive and specific safety biomarkers. Integration of multiple platform technologies including LC/MS/MS, LTQ-Orbitrap, ELISA, and Luminesx is used to analytically and biologically validate safety biomarkers throughout drug development. Limiting antibody reagents across preclinical species for a particular assay can be overcome by employing MS technologies strategically. TFF5 is one safety biomarker for renal (urine) and GI (serum) injury where multiple platform technologies have been employed to analytically validate biomarker values. These approaches and technologies can be applied during clinical trials and are feasible for such as HTN use, whereas antibody technology shows limitations in cross-validations across species. Lead optimization approaches are employed to utilize safety biomarkers early in the pipeline using samples from discovery PK studies. Lead optimization allows chemist support to be maximized in the pipeline, while screening for known toxicity liabilities. Prodrum renal functional markers of early organ toxicity are presented in a lead optimization format that is earlier than traditional histopathology endpoints. The renal model utilizes bile acids, leukotriene and proximal tubule enzymatic activity. It is critical that the biomarkers developed for use lead optimization are highly applicable to several preclinical models.

1445 TOXICITY TESTING IN THE 21ST CENTURY FOR ECOTOXICOLOGY.


The National Research Council (NRC) report, Toxicity Testing in the Twenty-first Century: A Vision and a Strategy has relevance for ecological as well as human health risk assessment. In April 2009, the Society of Environmental Toxicology and Chemistry (SETAC) held a workshop that considered key elements of the scientific foundation that would be needed to implement the vision of toxicity pathway-based testing in support of ecological risk assessment. The term adverse outcome pathway (AOP) was used to describe the linkage of molecular events modeled in a toxicity pathway assay to downstream biologic effects considered adverse from an ecotoxicological perspective (i.e., effects on survival, reproduction). Five challenges facing the application of this approach in this field, and attempt to provide a solution to these challenges.

1446 ADVERSE OUTCOME PATHWAY (AOP) MODELING OF KNOWN PATHWAYS.

M. E. Anderson1, K. Watanabe2 and R. Schulke2, 1The Hamner Institute, Research Triangle Park, NC, 2Oregon Health and Science University School of Medicine, Portland, OR and Battelle Pacific Northwest, Sequim, WA.

A nine-member workgroup representing disciplines of neurotoxicology, wildlife biology, ecotoxicology, and engineering developed a case study for domoic acid, an algal toxin with adverse effects on both wildlife and humans. The case study demonstrated strategies to develop computational models of known AOPs in an effort to move beyond the current toxicity testing paradigm focused on chemical-specific toxicity to a focus on toxicity pathway perturbations applicable for ecological risk assessment. Domoic acid is a potent agonist for kainate receptors - iotonic glutamate receptors whose activation leads to the influx of sodium and calcium. Increasing intracellular calcium concentrations lead to toxicity and cell death. The development of a conceptual framework for the AOP required an iterative process with two important outcomes: (1) a critically reviewed pathway from exposure to adverse outcome that is stressor specific and (2) identification of a key cellular process (or processes) suitable for evaluation in a mechanistic assay in vitro. The toxicity pathway indicated that in vitro cellular assays of altered neuronal calcium should serve as a measure of the key response and that the results of these assays would be amenable to mechanistic modeling for identifying perturbations (and domoic acid treatments) that are within normal, those that are adaptive, and those that are clearly toxic. In vitro assays with outcomes that are also amenable to measurement in exposed populations would link in vitro to in vivo conditions. Toxicokinetic information with domoic acid also aids in linking in vitro results to the individual. Another required step is taking projected responses of individuals and creating population models. All these linkages were considered in group consideration of the specific domoic acid example, leading to a series of more specific recommendations about strategies for literature mining and model development for known pathways.

1447 REVERSE ENGINEERING ADVERSE OUTCOME PATHWAYS FROM ‘OMICS DATA.

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While many toxicologically-relevant pathways are known, many responses are mediated via unknown, or poorly characterized, mechanisms and modes of action. The advent of global analysis tools provides new capabilities for probing entire biological systems. Complex interaction networks can be reverse engineered or inferred from gene, protein, metabolic, signaling data enabling exploration of potential modes or mechanisms of toxic action. We describe reverse engineering of interaction networks from genes, proteins, and metabolites altered by toxicants to identify adverse outcome pathways in ecotoxicology. We then examined the utility of this for deducing toxicologically-relevant networks that regulate response to a defined stressor and dictate outcomes. A large data set of 868 arrays was used that focused on expression changes in fathead minnow ovary tissue representing exposure to 7 different chemicals, over different times, and in vivo versus in vitro conditions. By applying different approaches, we demonstrate how this data set can be used to infer gene regulatory networks. The network path from stressor to adverse outcome can be considered a candidate adverse outcome pathway. Identification of candidate adverse outcome pathways allows for the formation of testable hypotheses about the key biologic processes, biomarkers or alternative endpoints, which could be used to monitor an adverse outcome pathway. Finally, we identify the unique challenges facing the application of this approach in this field, and attempt to provide a road map for the utilization of these tools.
The viability of populations of plants and animals is a key focus for environmental regulation. Population-level responses integrate the cumulative effects of chemical stressors on individuals as those individuals interact with and are affected by their co-species, competitors, predators, prey, habitat and other biotic and abiotic factors. Models of population-level effects of contaminants can integrate information from lower levels of biological organization and feed that information into higher-level community and ecosystem models. As individual-level endpoints are utilized to predict population responses, this requires that biological responses at lower levels of organization be translated into a form that is useable by the population model. In this presentation we describe how mechanistic data, as captured in adverse outcome pathways, can be translated into modeling focused on population-level risk assessments. First, we provide a succinct overview of different approaches to population modeling, and discuss the types of data needed for these models. Then we discuss how toxicity data are used currently for population modeling, and provide recommendations as to how testing might be modified to better generate information to support modeling. From this we describe how different key processes measured at the level of the individual serve as the bridge between mechanistic toxicology data and predictions of population status, and provide case examples of how this linkage has been/can be achieved.

Safety factors are used in ecological risk assessments to extrapolate from the toxic responses of a limited number of laboratory test species to all species representing that group in the environment. Advances in understanding the mechanistic basis for toxicological responses can provide a biological basis to more accurately extrapolate across species and, in part, an explanation for the variability in whole organism responses to toxicants. We highlight potential short and medium term goals in the direction of truly predictive in silico extrapolation across species’ response to toxicants, and present a conceptual approach. Critical information is required to establish evidence-based extrapolation including the identification of critical molecular pathways and regulatory networks that are linked to the biological mode of action. A case study is presented that examines steroidogenesis inhibition in fish following exposure to the aromatase inhibitors fadrozole or prochloraz. Extremely similar effects for each compound among fathead minnow, medaka, and zebrafish were attributed to similar inhibitor pharmacokinetic/pharmacodynamic distributions and similarities in the sequences of CYP19A1 and CYP19A2. Rapid advances in homology modeling allow the prediction of chemical interactions with enzymes, making it possible to eventually allow a prediction of aromatase inhibitor effects of new compounds across a range of species. Critical knowledge gaps include differences in life histories (e.g., reproductive strategies), tissue specific gene expression and the role of metabolism on toxic responses.

Animals have evolved a variety of mechanisms for adapting to toxic chemicals of both natural and anthropogenic origin. From a regulatory perspective, these adaptive responses compound efforts to establish acceptable levels of chemical exposure. To illustrate this point, we consider endocrine systems as targets for environmental contaminants. Existing data suggest that these systems possess a robust capacity to adapt to and recover from chemical effects. Using the hypothalamic-pituitary-thyroid (HPT), -gonad (HPG), and -adrenal (HPA) axes as case examples, we highlight system attributes that provide for this adaptive capability. In doing so, a distinction is made between effects on adults and developing organisms. Emerging data indicate that endocrine system disruption during early development may lead to changes in epigenetic programming, resulting in permanent changes in phenotype. Risk assessments of chemicals that impact highly regulated systems must consider the dynamics of these systems in relation to complex environmental exposures. Mechanistically-based mathematical models of endocrine systems provide a means for better understanding adaptation and recovery. In the short term, these models can be used to design experiments and interpret study findings. In the longer term, a set of validated models could be used to extrapolate limited in vitro and in vivo testing data to a broader range of unstressed chemicals, species, and exposure scenarios. With appropriate modification, Tier-2 assays developed in support of the U.S. EPA’s Endocrine Disruptor Screening Program could be used to assess the potential for adaptation and recovery, and inform the development of mechanistically-based models. This abstract does not necessarily reflect U.S. EPA policy.
reported for endocrine disrupting chemicals thought to exert health effects primarily by interfering with steroid hormone receptor function. Participants will learn the latest details of steroid hormone action including binding of endogenous hormone and/or hormone mimics to steroid receptors and the possibility of non-receptor-mediated actions. This will set the stage for examining mechanisms by which low levels of exogenous ligand may alter endogenous responses or developmental processes. With this foundation, diverse effects reported for the weak estrogenic compound Bisphenol A will be reviewed, particularly surprises seen at very low doses where the dose-response deviates from the expected linear relationship. Recent explorations of gene regulatory networks suggest explanations for these unexpected nonlinearities, especially at very low levels of signal. Discussion will add perspectives on the mechanistic and risk assessment implications provided by the new science of steroid signaling and gene regulatory networks. By providing this information, we hope that the audience will come away from this session with a better understanding of the biological plausibility of low dose effects of endocrine disrupting chemicals as well as implications for risk assessment and predictive toxicology. Additionally, participants will gain insights on how to focus on nonlinear biological networks, as opposed to our accustomed linear pathways, and how to leverage all available data to detect very low dose effects into outcomes more conventionally measured in toxicity studies.

1453 ESTROGEN RECEPTORS AS SENSORS AND MEDIATORS OF DIVERSE LIGAND RESPONSES.
G. L. Greene, The Ben May Department for Cancer Research, University of Chicago, Chicago, IL; Sponsor: S. Darney.

Natural and synthetic ligands regulate diverse signaling and transcriptional networks via one or both of two estrogen receptor subtypes (ERα & ERβ). The structural and ligand-specific recruitment of coregulators and modulation of target gene expression have important implications for understanding the specificity of nuclear receptor signaling, for the treatment or prevention of a variety of diseases, and for endocrine disruption. The development of diverse ER subtype-selective ligands provides molecular tools to study unresolved issues in the structural linkage between ligand and transcription. To better understand the relationship between nuclear receptor ligand positioning and the formation of co-factor-binding surfaces, we have solved multiple ERα/lBD structures and investigated some of the determinants of ligand selectivity between the two estrogen receptor subtypes. Structurally guided amino acid substitutions have identified amino acids required for selectivity. Residues within the ligand-binding pocket as well as distal secondary structural interactions contribute to subtype specific positioning of the ligand and coregulator selectivity. These data demonstrate the importance of both short- and long-range interactions in the allosteric transmission of information through the nuclear receptor ligand-binding domain, and in determining the specificity of closely related receptor subtypes, such as ERα & ERβ. Detailed structure-function information for the two ERs has proved useful both for understanding as well as designing ligands with tissue- and pathway-selective behaviors. Co-occupancy of receptor dimers by ligand mixtures as well as occupancy of a single monomer by ligands at low doses may contribute to the unexpected behavior of some endocrine modulators.

1454 LOW DOSE EFFECTS OF BISPHENOL A IN ANIMAL STUDIES.
K. Thayer and J. Bucher, NIEHS, Research Triangle Park, NC.

The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) evaluated bisphenol A (BPA) and released its NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of BPA in 2008. NTP’s evaluation included an analysis of nearly 1,000 studies in experimental animals and in vitro model systems. Many of the animal studies that explored effects of exposure to “low” doses of BPA during critical periods of development reported effects that would not have been predicted from the “high” dose literature. Collectively, the low dose findings could neither be completely explained, nor could they be dismissed. NTP assessed the biological plausibility of these effects in the context of consistency with BPA as an “estrogenic” compound. It concluded that in many cases the effects occurred at doses that were too low to have predicted simply on the basis of relative potency to typical positive control compounds like DES or 17β-estradiol. These observations were especially intriguing in light of in vitro data suggesting that BPA may exert effects through non-estrogen receptor pathways. For example, BPA was more active than other chemicals tested in the EPA ToxCast screening of Endocrine Disruptor Screening Program priority chemicals, with 111 “hits” from 467 assays. Also, BPA appears to be interacting with non-classical estrogen receptors in patterns that differ from positive estrogenic controls, i.e., ERα, GPR30, and non-classical membrane estrogen receptor. NTP also considered the hypothesis that BPA exerts different effects at “high” and “low” doses, i.e., non-monotonic dose-response. However, in most instances the existing literature was inadequate to thoroughly evaluate this hypothesis because the studies that reported low-dose effects of BPA often did not test multiple dose levels, and the studies that tested a wide range of doses generally did not report response deviations from the expected linear dose response. In many cases, particularly for findings based on studies with very specific experimental questions, variations in experimental design are large enough to conclude that the reproducibility of the low-dose findings is essentially unknown.

1455 DOSE-RESPONSE RELATIONSHIPS IN GENE REGULATORY NETWORKS AND LACK OF LINEARITY AT LOW DOSE.
Q. Zhang, The Hamner Institutes, Durham, NC; Sponsor: M. Andersen.

Many environmental chemicals produce toxicological outcomes by affecting a multitude of responsive gene regulatory networks operating in the cell. These gene networks are responsible for a variety of cellular functions including detoxification, homeostasis, proliferation, differentiation, and apoptosis, etc. It is imperative to examine the quantitative aspects of the gene regulatory networks that are responsive to endocrine disrupting chemicals (EDCs) since they determine the ultimate disrupting consequences to the endocrine system. With respect to risk assessment for EDCs, two fundamental molecular processes are at work in the cell: 1) adaptive activation of the metabolic gene network responsible for detoxifying the disrupting chemicals entering the cell, and 2) activation/inhibition of the gene networks responsible for the synthesis, metabolism, and action of the endogenous steroid hormones. By forming a negative feedback loop and employing ultrasensitive signaling motifs, the inducible metabolic gene network is likely to generate an inherently nonlinear intracellular concentration of EDCs. Subsequently, the intracellular EDCs may act as steroid agonist or antagonist via interacting with steroid hormone receptors to affect expression of target genes, usually in the presence of the endogenous ligands. Because the steroid hormone receptors dimerize after ligand binding, it may lead to nonmonotonic gene expression changes in response to EDCs acting as agonists. Lastly, since hormone homeostasis of endocrine systems is often maintained via long negative feedback loops involving the hypothalamus and pituitary, EDCs that affect the synthesis and metabolism of endogenous hormones likely cause nonlinear changes to the concentrations of these hormones. Together, the theoretical works suggest that the disrupting consequences of EDCs mediated through the operation of responsive gene networks are likely to be nonlinear, especially at low doses.

1456 THE KINETICS OF URINARY FUMONISIN EXCRETION IN HUMANS CONSUMING MAIZE-BASED FOODS.
R. T. Riley1,2, Toxicology and Mycotoxicosis Research Unit, USDA-ARS, Athens, GA and 2Interdisciplinary Toxicology Program, University of Georgia, Athens, GA.

Fumonisins (FB) are mycotoxins found in maize worldwide. In Central America, South America and Mexico, maize-based foods are often a major part of the diet and FB intake can also be high. A study in Mexico found a significant correlation between urinary FB1 and maize tortilla consumption. The purpose of this study was to determine the relationship between FB intake and excretion in humans consuming maize-based foods. Maize-based foods were prepared from local stores and analyzed for FB. All maize-based foods tested were positive for FB ranging from 18 ppb to 8 ppm. Commercial Maize flour and products containing predomi- nantly maize flour often contained 1 to 3 ppm of FB. Maize tortillas and maize-biscuits were prepared from these two products and 200 grams (six tortillas and five biscuits) were consumed by a single volunteer for three days. Urine samples were collected at each urination and total urine volume was recorded. This was also done for three days before and for five days after consuming the tortillas and biscuits. Seven additional volunteers consumed tortillas and biscuits but only collected a single urine sample every 24 h over 11 days of the study and provided estimates of total urine output each day. The urine was analyzed for FB1, FB2, FB3 and hy- droyzed FB1, FB2 and FB3. In all of the urine samples analyzed only FB1 was detected. Excretion in urine peaked at or soon after day 3 of consuming and 24 h later.
the level in urine was 25% of the maximum level and within five days after con- 
sumption ended FB1 was no longer detectable in the urine. The total amount of 
FB1 excreted in the urine was less than 1% of the total intake. The results indicate 
that the use of urinary FB1 as a marker to assess ongoing exposure in a population 
based study is possible but relating urinary levels to levels of exposure could be 
difficult due to individual variability and the rapidity of clearance.

**1457 INITIATION OF GLOBAL NETWORK FOR AFLATOXIN EXPOSURE STUDIES**

L. Wang, G. Qian, L. Xu, N. Johnson, P. Jolly, J. H. Williams, T. D. Phillips, and J. S. Wang. The University of Georgia, Athens, GA; Texas A&M University, College Station, TX; and University of Alabama at Birmingham, Birmingham, AL.

Aflatoxins (AF) are a group of mycotoxins and significant food contaminants, espe- 
cially in peanuts and corn. AFBI is hepatotoxic and genotoxic, and a known human carcinoma (Group I). Acute exposure to high levels of AFBI causes aflatoxi- 
cosis and death in humans. Chronic exposure to low levels of AF is a major risk 
factor for human liver cancer. AFBI is also a potent immunotoxic agent which is 
linked to high rate of infectious diseases in developing world. Therefore, accurate 
assessment of global AF exposure is urgently needed. In this project, we initiated a 
global network to study AF exposure using levels of serum AFBI by a sensitive 
immune assay (AFBI-lys) as a biomarker. We measured serum samples collected from the San 
Antonio (SA) Environmental Health Study (n=172), a case-control study in 
Guangxi controls averaged 7.34 \pm 90.7% in Ghanaian mothers, 79% in Ghanaian infants, and 76.2% in Haitians, re-

**1459 CLINICAL INTERVENTION WITH NOVASIL CLAY REDUCES FUMONISIN B1 EXPOSURE IN A WEST AFRICAN POPULATION**

A. Robison1, N. M. Johnson1, A. Streys1, J. F. Taylor1, A. Marroquin-Cardona2, E. Afriyie-Gyau1, R. Nachmani2, N. A. Arkanah1 and T. D. Phillips1. College Vet Med, Texas A&M University, College Station, TX; USDA/ARS, College Station, TX; 1Georgia Southern University, Statesboro, GA and 2NMIMR, University of Ghana, Accra, Ghana.

Chronic exposure to fumonisin B1 (FB1), a hazardous fungal contaminant fre- 
quently found in corn and other staple foods, has been correlated with an increased 
incidence of esophageal cancer and neural tube defects in humans. Additionally, 
FB1 has been implicated as the causative agent in leukoencephalomalacia and 
porcine pulmonary edema in horses and swine, respectively. FB1 may act to en-
hance aflatoxin (AF) carcinogenicity due to its activity as a cancer promoter and fre-
quent co-contamination with AFs in food. Recent literature indicates urinary 
FB1 is a sensitive biomarker of exposure. Thus, the major objectives of this study 
were 1) to utilize urinary FB1 as a biomarker for FB1 exposure; 2) to assess poten-
tial co-exposures in a West African population highly exposed to AFs and FB1; and 
3) to evaluate the efficacy of Novasil (NS) as an intervention strategy for FB1 ex-
posure. Urine samples (n = 118) were collected from participants in the Ejeua-
Sckyedumase district of Ghana, and fumonisin exposure was evaluated using ur-
inary FB1 as a biomarker. Mean quantities of urinary FB1 were 5.03, 0.72, and 0.81 
ng FB1/ml urine in the placebo (cellulose, 1.5 g/day), low dose (NS, 1.5 g/day), 
and high dose (NS, 3.0 g/day) treatment groups, respectively. Based on this, 
urinary FB1 was verified vs HPLC separation and fluorescence detection to be a 
sensitive biomarker of FB1 exposure. Additionally, this study demonstrates that 
Novasil treatment significantly reduces exposure to FB1. Frequent and concurrent 
exposure to aflatoxin and fumonisin in the diet may facilitate the toxic, carci-
ogenic, and teratogenic effects of these chemicals. Importantly, Novasil may act as a 
multifunctional enteroabsorbtion for acute and chronic exposures to FB1 and AFBI 
and significantly impact the health of populations, both human and animal, at risk 
for mycotoxin induced morbidity and mortality.

**1460 SUPPRESSIVE EFFECT OF PECTIN GELATION ON ABSORPTION OF DEOXYNIVALENOL IN MICE**

Y. Sugita-Konishi1, D. Koyama2, T. Kadota2, S. Itoh2, K. Sugiyama2, C. Tamura1, M. Nishijima3 and Y. Kamata1. 1Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan, 2School of Veterinary Medicine, Azabu University, Kanagawa, Japan, 3Kerin Holdings Company Limited, Gunma, Japan and 4Department of Food and Health Sciences, Jissen Women’s University, Tokyo, Japan.

Mycotoxin is natural contaminant and cause adverse effects on human and animal 
health. Since it is too hard for mycotoxin to reach zero tolerance, many approachs 
to reduce intake mycotoxin from final food products have been tried. However the 
developed tools until now has advantages and disadvantages. Therefore the develop-
ment of new method for reduction of exposure to mycotoxin is needed on food 
processing. Pectin is constituent of plant, and has been used in the food industry as 
a natural ingredient. It has ability to form gels at low concentrations. Pectin is di-
versely found in corn and other staple foods, has been correlated with an increased 
incidence of esophageal cancer and neural tube defects in humans. Additionally, 
FB1 has been implicated as the causative agent in leukoencephalomalacia and 
porcine pulmonary edema in horses and swine, respectively. FB1 may act to en-
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FB1 has been implicated as the causative agent in leukoencephalomalacia and 
porcine pulmonary edema in horses and swine, respectively. FB1 may act to en-
hance aflatoxin (AF) carcinogenicity due to its activity as a cancer promoter and fre-
quent co-contamination with AFs in food. Recent literature indicates urinary 
FB1 is a sensitive biomarker of exposure. Thus, the major objectives of this study 
were 1) to utilize urinary FB1 as a biomarker for FB1 exposure; 2) to assess poten-
tial co-exposures in a West African population highly exposed to AFs and FB1; and 
3) to evaluate the efficacy of Novasil (NS) as an intervention strategy for FB1 ex-
posure. Urine samples (n = 118) were collected from participants in the Ejeua-
Sckyedumase district of Ghana, and fumonisin exposure was evaluated using ur-
inary FB1 as a biomarker. Mean quantities of urinary FB1 were 5.03, 0.72, and 0.81 
ng FB1/ml urine in the placebo (cellulose, 1.5 g/day), low dose (NS, 1.5 g/day), 
and high dose (NS, 3.0 g/day) treatment groups, respectively. Based on this, 
urinary FB1 was verified vs HPLC separation and fluorescence detection to be a 
sensitive biomarker of FB1 exposure. Additionally, this study demonstrates that 
Novasil treatment significantly reduces exposure to FB1. Frequent and concurrent 
exposure to aflatoxin and fumonisin in the diet may facilitate the toxic, carci-
ogenic, and teratogenic effects of these chemicals. Importantly, Novasil may act as a 
multifunctional enteroabsorbtion for acute and chronic exposures to FB1 and AFBI 
and significantly impact the health of populations, both human and animal, at risk 
for mycotoxin induced morbidity and mortality.
**1461 FORMATION OF MIXED ADDUCTS WITH GLUTATHIONE AND ADENINE: A POSSIBLE CONTRIBUTION TO THE MUTAGENICITY OF THE MYCOTOXIN PATULIN.**

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The mycotoxin patulin (PAT) is a frequent contaminant in apple juice. Most products contain less than 50 μg/l (0.3 μM); however, some reach up to 1 mg/l. Based on the provisional maximum tolerable daily intake established by the WHO, 50 μg/l is considered to be safe. Recently, we have shown the mutagenic potential of PAT in the hprt locus of V79 cells at concentrations as low as 0.6 μM. Furthermore, we demonstrated that in this concentration range, DNA-DNA crosslinks rather than DNA strand breaks or oxidative DNA base modifications contributed to the PAT-induced DNA damage. The mutagenicity of PAT at such low concentrations is in contrast with the assumption that the intracellular excess of glutathione (GSH) ascertains the complete inactivation of small reactive electrophiles such as PAT. Therefore, we investigated the reactivity of PAT towards the DNA base adenine (A) in the presence and absence of equimolar concentrations of GSH in vitro. The reaction products of (i) GSH+PAT, (ii) PAT+A, and (iii) PAT+A+GSH were separated by HPLC and their structure was elucidated by mass spectrometry (ESI plus, triple quadrupole). In addition, adducts of PAT with GSH were verified by characterized standard compounds. As expected, reaction of PAT with GSH at room temperature for 3 days produced a multitude of products exhibiting previously identified structures containing 1-3 GSH moieties. Moreover, reaction of PAT with A yielded several novel products. In the presence of A and GSH together, most of the known GSH-PAT adducts were detected, albeit at different quantities. Whereas some products observed after reaction of PAT with A alone were not detectable in the presence of GSH, at least one PAT-A adduct persisted. In addition, at least one new product exhibiting a m/z ratio and mass spectrum suggesting a mixed GSH-PAT-A adduct with a ketocarboxylic acid backbone was detected. Thus, the present study demonstrates the reactivity of PAT towards a DNA base not only despite the presence of GSH but also involvement of GSH in adduct formation.

**1462 INDIVIDUAL AND COMBINED EFFECTS OF THE MAIN FUSARIUM TOXINS IN THE MOUSE AFTER 7 DAYS TREATMENT.**

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Male and female Swiss mice (n=5/group) were treated by oral route for 7 consecutive days with fumonisin B1 (FB1), zearalenone (ZEA) and the deoxynivalenol (DON) at doses of 110 μg/kg bw/day, 50 μg/kg bw/day and 45μg/kg bw/day respectively. The biological analyses of blood and the urines showed that (i) triglycerides increase by more than 20 to 50% with FB1 and especially with DON and with the combinations of the three toxins. (ii) The plasmatic proteins increase significantly following the treatment by FB1 and the combinations containing FB1 but also with combination of ZEA+DON whereas taken individually each one of these toxins does not have this effect. (iii) The creatinuria increases, accompanied by a decrease of the creatinineuria for FB1 and its combinations just as for ZEA+DON suggesting renal damage induced by these treatments. Analysis of the organs at cellular and molecular levels shows (i) the presence of modified bases in main tissues (liver and kidneys) with all toxins taken individually or in combination. (ii) DON induces in renal tissues a fragmentation of the ADN with DNA ladder shape, which is typical of apoptosis suggesting a possibility of reduction of the weight of this organ by a prolonged treatment with DON as it was found for FB1 although the mechanisms seem different. As observed in vitro, the combinations of these toxins of Fusarium induce always more lesions than toxins taken individually suggesting a revision of the current lawful limits in the cases of multi contamination by the mycotoxins.

**1463 DEOXYNIVALENOL-INDUCED MODULATION OF MICRNARNA EXPRESSION IN RAW 264.7 MACROPHAGES: A POTENTIAL NOVEL MECHANISM FOR TRANSLATIONAL INHIBITION.**

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MicroRNAs (miRNAs) are short oligonucleotides that influence various biological processes by binding to the target genes 3’-UTRs thereby facilitating suppression of translation and/or mRNA decay. The objective of this research was to study how the trichothecene mycotoxin deoxynivalenol (DON) influences the endogenous miRNA profile in RAW 264.7 macrophages and predict their potential regulatory roles on ribosomal protein miRNA expression using the miRNA database microCosm. RAW 264.7 cells were treated with 250 ng/ml DON for 2 h and 6 h, and RNA analyzed by RT-PCR array to measure changes in expression of 376 known miRNAs. Clustering analysis revealed that changes in expression of miRNAs was observed both at 2 h and 6 h, but that there were more distinct up-regulated miRNAs observed at 6 h. The data showed that 91% of all the ribosomal protein miRNAs could be potentially regulated by miRNAs. The large subset ribosomal proteins (RPLs) with predicted miRNA upregulation were very similar at 2 and 6 h (90% and 93%, respectively), while those with downregulated miRNAs decreased from 46% at 2 h to 23% at 6 h. The small subunit ribosomal proteins (RPSs) showed a similar trend to RPLs with upregulated miRNA increases from 79% at 2 h to 96.5% at 6 h s and downregulated miRNAs of 48% at 2 h and 27% at 6 h. The results suggest that downregulation of ribosomal protein miRNAs could conceivably contribute to the known capacity of DON and other trichothecenes to inhibit protein translation.

**1464 POTENTIAL CYTOTOXIC EFFECTS OF FUSARIUM MYCOTOXINS IN OVARIAN AND KIDNEY CELL LINES.**

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Beauvericin (BEA), deoxynivalenol (DON) and the T-2 toxin belongs to the large group of mycotoxins synthesized by various Fusarium moulds are known to coexist in certain food sources, such as cereals. Mycotoxins pose a potential health risk in human nutrition. Several acute and chronic toxic effects have been observed in humans after consumption of contaminated food. The aim of this study was to determine the individual and combined cytotoxic effects of BEA, DON and T-2 toxin in ovarian and kidney cell lines after 24, 48 and 72 h exposition. With an IC50 value ranged from 2.33 to 3.22 μM (in CHO-K1 cells) and from 3.75 to 5.31 μM (in Vero cells), T-2 toxin showed the strongest cytotoxic effect in both cell lines. DON revealed weaker cytotoxic effects with an IC50 ranged from 0.51 to 0.60 μM (in CHO-K1 cells) and from 3.30 to 10.00 μM (in Vero cells). BEA was the less cytotoxic with an IC50 value ranged from 1.67 to 2.17 μM (in CHO-K1 cells) and from 6.77 to 11.08 μM (in Vero cells). When mycotoxins are simultaneously present, the cytotoxicity was considerably enhanced. The synergistic effect order observed after mycotoxin combinations could be established as follow: T-2 toxin + BEA + DON > T-2 toxin + BEA > T-2 toxin + DON > BEA + DON, with the T-2 toxin being the most potently interactive compound. In conclusion, T-2 toxin, DON and BEA are able to induce cytotoxic effects in ovarian and kidney cells and thus evoke additive or synergistic effect depending on the concentration used and time of exposure. From the mycotoxicological point of view due to the potent toxic effects of BEA, DON and T-2 toxin, simultaneous exposure to those mycotoxins might be an important trigger for development of several diseases in humans, especially after long-time exposure. This work was supported by the Spanish Science and Education Ministry (AGL2007-61493).

**1465 PDK-1 AND THE AKT-1/2 COMPLEX REGULATE C. ELEGANS METALLOTHIONEIN TRANSCRIPTION IN RESPONSE TO CADMIUM.**

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Humans are constantly exposed to the carcinogenic metal cadmium through various environmental routes. Cadmium accumulates in cells and induces oxidative stress, alters protein activity, inhibits DNA repair, and activates various cellular response pathways. In response to cadmium, cells increase the expression of highly conserved cysteine-rich metal-binding proteins known as metallothioneins (MTs) that function in metal detoxification and homeostasis. The nematode C. elegans has two MT genes: mtl-1 and mtl-2. To identify regulatory factors and pathways that control metal-inducible mtl-1 transcription, integrated transgenic strains of C. elegans containing GFP under the control of the 5’-regulatory region of mtl-1 were constructed, pmtl-1::GFP. The transgenic strains constitutively expressed GFP in the pharynx and following cadmium exposure GFP expression was observed in the intestine. Using the pmtl-1::GFP strain, genes involved in the intestine. Using the pmtl-1::GFP strain, genes involved in the metal homeostasis, aging/stress response and oxidative stress pathways were tested to see if they affected metallothionein expression. In response to cadmium, MT gene expression was increased. Using the pmtl-1::GFP strain, genes involved in the metal homeostasis, aging/stress response and oxidative stress pathways were tested to see if they affected metallothionein expression. In response to cadmium, MT gene expression was increased. Using the pmtl-1::GFP strain, genes involved in the metal homeostasis, aging/stress response and oxidative stress pathways were tested to see if they affected metallothionein expression. In response to cadmium, MT gene expression was increased. Using the pmtl-1::GFP strain, genes involved in the metal homeostasis, aging/stress response and oxidative stress pathways were tested to see if they affected metallothionein expression. In response to cadmium, MT gene expression was increased.
The farnesoid X receptor (FXR) is a member of nuclear receptor superfamily. FXR is highly expressed in the liver and intestine. Previous studies have implicated beneficial functions of FXR in the homeostasis of bile acids, lipids, and glucose, as well as in liver regeneration and carcinogenesis. To further investigate the role of FXR in vivo, we generated transgenic mice that bear the activation of FXR in the liver. Consistent with previous reports, activation of FXR in transgenic mice induced FGF15 and suppressed CYP7A1 gene expression, as well as decreased circulating and hepatic levels of triglycerides. Interestingly and surprisingly, the FXR transgenic mice showed spontaneous hepatotoxicity and signs of increased inflammation as in liver regeneration and carcinogenesis. To further investigate the role of FXR in daily light/dark cycles. Many xenobiotic metabolizing genes are reported to be expressed in a coordinated daily rhythm. We have reported significant daily variation in susceptibility to pesticide exposure in male Drosophila melanogaster. Here we examine how the circadian clock modulates the underlying molecular rhythms contributing to sex-specific daily susceptibility profiles. Flies were maintained in 12h:12h light/dark conditions, or moved to constant darkness or light prior to testing. Flies were acutely exposed to a series of doses of propoxur, malathion, deltamethrin, or fipronil for one hour, every four hours for 24 hours, and scored for mortality two days later. In a 12h:12h light/dark regime, LC50 of females was 100 fold that of males at ZT4 in response to propoxur. In constant light, which disrupted the circadian clock, the daily rhythm in susceptibility previously observed in males in LD was flattened, and average LC50 across the day little reduced. In females, the LC50 across the day was reduced five fold in constant light. To examine which arm of the circadian clock is responsible for this effect, we demonstrated that defects in the central clock genes Pdp1 and CLK/CYC lead to increased susceptibility to pesticides in male flies by modulating the expression of CAR. We are currently examining sexual dimorphism in daily susceptibility to pesticides in flies with mutations in Pdp1, CLK/CYC, and PER. We are also comparing expression differences in clock genes, nuclear receptors, and xenobiotic metabolizing genes between males and females using enzyme activity and qRT-PCR. This work details mechanisms of circadian clock modulation of sexual dimorphism in xenobiotic metabolism in Drosophila. Importantly, this work reveals potential interactions between chemical exposure and disruption of the circadian clock, which is associated with disease in humans.
The aryl hydrocarbon receptor (AhR) is traditionally recognized for its role in the adaptive metabolism of xenobiotics, but has more recently been associated with important physiological processes such as cell cycle regulation and apoptosis. Our lab has recently followed up on observations suggesting that the AhR is able to promote cell survival or death in response to intrinsic or extrinsic apoptotic stimuli, respectively. Using primary hepatocytes isolated from the AhR+/- mouse, we generated AhR null hepatocytes through infection with an adenovirus expressing the Cre recombinase. Complete loss of AhR expression occurred within 24 hours. AhR expression was unaltered in control cultures infected with a control adenovirus. Susceptibility to UV-irradiation induced apoptosis was assayed by measuring caspase-3 activity, and monitoring chromatin condensation and cell blebbing. Since Akt activity was recently implicated in promoting AhR-mediated Hepa-1 cell survival upon intrinsic stimulation, we examined Akt expression and activity in the primary hepatocytes immunologically. Our findings confirmed that the AhR indeed promotes survival, but does so in a mechanism independent of Akt activity. In order to ascertain how the AhR functions to protect primary hepatocytes from intrinsic cell death, we performed a detailed DNA microarray analysis linking changes in the transcriptome directly to the loss of AhR expression—in the absence of an exogenous receptor agonist. This screen represents a unique strategy for assessing the receptor’s physiological role in regulating gene expression, and identified a distinct set of genes with altered expression not previously associated with AhR activity. In order to ascertain how the AhR functions to protect primary hepatocytes immunologically. Our findings confirmed that the AhR indeed promotes survival, but does so in a mechanism independent of Akt activity. In order to ascertain how the AhR functions to protect primary hepatocytes from intrinsic cell death, we performed a detailed DNA microarray analysis linking changes in the transcriptome directly to the loss of AhR expression—in the absence of an exogenous receptor agonist. This screen represents a unique strategy for assessing the receptor’s physiological role in regulating gene expression, and identified a distinct set of genes with altered expression not previously associated with AhR activity. As a result, we are now in position to characterize new AhR-dependent target genes and mechanisms, including protection from apoptosis, consistent with normal AhR physiology. Supported by ES01218 and T32ES07254.

**1472 FEEDBACK LOOP BETWEEN RETINOIC ACID RECEPTORS AND NOVEL COREGULATOR TNIP1.**


Transcriptional control by nuclear receptors (NRs) is mediated not only through their ligands, but also through coregulator proteins which act either as coactivators or corepressors of NR activity. In a yeast two-hybrid screen seeking novel NR coregulators, we isolated TNIP1, a protein previously reported to interact with two different HIV proteins and also to repress NF-kb activity. TNIP1 amino acid sequence revealed the presence of two LXXLL NR box motifs suggesting it may be a coregulator. TNIP1 does not interact with retinoid X receptor (RXR) but associates with retinoic acid receptors (RAR) α and γ in a ligand dependent fashion and exhibits other interaction hallmarks of a coactivator. However, TNIP1 represses RAR activity in the presence of ligand. This makes TNIP1 an unusual coregulator—a co-repressor of agonist bound NRs. The repression is partially relieved by coactivator SRC3, suggesting interference with coactivator recruitment as a mechanism of TNIP1 action. TNIP1 does not associate with histone deacetylase (HDAC) enzymes, suggesting the TNIP1 repression is HDAC independent. With this atypical function, we sought to determine what might contribute to control of TNIP1 expression. We carried out in silico analysis of its promoter to identify putative transcription factor binding sites which predicted several retinoic acid response elements. Transcriptional activation studies revealed that TNIP1 promoter is positively regulated by RARs. EMSA showed RAR binding at distinct sites in the distal portion of the TNIP1 promoter. Promoter-luciferase reporter studies confirmed these as response elements. Our findings reveal a potential regulatory feedback loop where TNIP1 expression is increased by RARs which, in turn, attenuates their activity. Such regulatory feedback loops between coregulators and their target NRs may serve to buffer cells against extremes of hormone-regulated signaling, such as the presence of toxic ligand levels, or cells being exposed to ligand in inappropriate times.

**1473 PROGRESSIVE EXPOSURE TO AMBIENT FINE PARTICLES IS ASSOCIATED WITH CHANGES IN PULMONARY AND SYSTEMIC INFLAMMATION IN MICE.**

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Growing evidence suggests exposure to particulate matter is associated with increases in morbidity and mortality due to cardiopulmonary complications. To examine for the presence of early markers of pulmonary and systemic inflammation, including changes in hematologic parameters, male Balb/C mice were progressively exposed to concentrated ambient particles (CAPS) in the fine/ultrafine size range or to filtered air in Fresno, CA during the summer of 2009 for 6 hours a day for 3, 6, 9, or 12 days. Total lung cells recovered by bronchoalveolar lavage (BAL) were significantly increased at 6 days, while significantly reduced at 12 days, compared with filtered air control values. Lung neutrophils were found to be increased with progressive CAPS exposure, attaining statistical significance at 12 days. Hematologic assessment demonstrated significantly increased white blood cells in the circulation at 6 days. These pulmonary and systemic findings provide further confirmation for a role of progressive exposure to concentrated inhaled fine/ultrafine particles to enhance the pro-inflammatory activity in the lungs along with secondary markers of cellular change in the systemic circulation of mice. Research funded by U.S. EPA RD 832414.

**1474 PARTIAL CHARACTERIZATION OF AMBIENT PM2.5 EXTRACT FROM SAHARAN DUST EVENTS.**

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Every year million of tons of African dust (Sahara) are transported into the Caribbean. As much as 8 million tons of African dust was estimated as reaching the Puerto Rican coast in one month alone (July 2000). This input of dust coupled with other natural and anthropogenic sources of particulate matter could impact air quality and intensify respiratory problems. The expression of interleukins in human bronchial epithelial cells (BEAS-2B) exposed to PM2.5 enriched with Sahara dust and its relation with oxidative stress was evaluated. PM2.5 Teflon filters were obtained from the PR Environmental Quality Board (EQB). Satellite information Tool for Modeling and Mapping Spectrometer (TOMS) aerosol index, in conjunction with EOBQ data, was combined to determine Sahara dust events (SDE). Filters were extracted for 24 hours in 175ml hexane/aceton (1:1). BEAS-2B cells were seeded onto a 96-well plate and incubated 48hrs prior exposure to the organic extracts. Attached cells were exposed for 24hrs at different PM extract concentrations (25, 50, 75 and 100 μg/ml) before being analyzed for biological response. Cell viability was assessed using the Neutral Red bioassay (Sigma). A direct relationship between extract concentration and cell viability was identified. Non-SDE extracts were not cytotoxic at concentrations less than 100 μg/ml. A direct dose relationship was also found between PM2.5 extract and IL-8 expression (Luminex, Millipore). With both SDE and Non-SDE organic extracts. However the magnitude of response was significantly higher for SDE extracts. Similar findings were observed with IL-6 expression but not highly significant. Preliminary results pointed to a decrease in glucose levels of SDE extracts, suggesting that components in Sahara dust might induce oxidative stress in BEAS-2B. Metals analyses on both extracts are currently being performed.

**1475 PULMONARY BIOASSAY STUDY WITH POTASSIUM TITANATE NONFIBROUS PARTICULATES (TERRACCESS JS) IN RATS.**

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Terraces JS particles are composed of nonfibrous potassium titanate (K2Ti6O15)- particle types and have been proposed for friction and filling applications. The aim of this study was to assess lung toxicity in rats exposed to potassium titanate particle types relative to positive and negative controls. Groups of male rats were intratracheally instilled with doses of 1 or 5 mg/kg of Terraces JS particles or α-t quartz particles. Phosphate-buffered saline (PBS) solution instilled rats served as vehicle controls. Following exposures, the lungs of PBS and particle-exposed rats were evaluated for bronchoalveolar lavage (BAL) fluid inflammatory biomarkers at post-inflammation time points of 1 week, 1 month, and 3 months lung histopathology at 1 month or 3 months. Pulmonary exposures to quartz particles produced sustained lung inflammation and significant cytoxic effects. In contrast, exposures to potassium titanate particles produced no significant lung inflammatory or cell-in-
jury effects when compared to controls. In addition, pulmonary exposures to quartz particles produced progressive, dose-dependent lung inflammatory responses characterized by neutrophils, foamy lipid-containing alveolar macrophage accumulation and early lung tissue thickening at the 3-month postexposure period. In contrast, exposures to potassium titanate particulates produced no significant adverse effects compared to controls, as evidenced by normal lung architecture at 1 and 3 months postexposure. The results demonstrate the benign nature of the pulmonary response in rats following particle exposures to 1 or 5 mg/kg (approximately 1.25 mg) of potassium titanate particle-types in these pulmonary bioassay studies. Thus, based on these results, it is concluded that inhaled Terraces JS particles are expected to have a low risk potential for producing adverse pulmonary health effects in exposed workers.

**1476 PLEURAL TRANSLLOCATION, FATE, AND PATHOLOGICAL RESPONSE OF CHRYSOTILE IN COMBINATION WITH FINE PARTICLES COMPARED TO AMOSITE ASBESTOS FOLLOWING SHORT TERM INHALATION EXPOSURE.**

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The pleural translocation, fate and pathological response of a commercial chrysotile similar to that which was used through the mid-1970's in a joint compound intended for sealing the interface between adjacent wall boards was evaluated following short-term inhalation exposure. Animals were exposed to sanded joint compound consisting of both chrysotile fibers and joint compound particles. The amphibole asbestos, amosite, was used as a positive control. Rats were exposed by inhalation 6 h/d for 5 days to a well-defined fiber aerosol. Sub-groups were examined through 1-year post-exposure. The translocation to and pathological response in the pleura was examined by SEM and confocal microscopy (CM) using non-invasive methods. The number and size of fibers was quantified using TEM and CM. This is the first study to employ such techniques to characterize fiber translocation to and the response of the pleural cavity. By 7 days following cessation of exposure, long amosite fibers were observed on the pleural membrane with a concomitant cellular response seen on the parietal pleural surface. The inflammatory response on the pleura had progressed by 90 days after cessation of exposure. The results from the non-invasive whole rat procedures identified well-developed surface granuloma in the pleural space of amosite exposed rats by 181 days after cessation of exposure. No cellular or inflammatory response was observed in the pleural cavity in response to the chrysotile and sanded material exposure. These results provide confirmation of the important differences between chrysotile mixed with sanded joint compound and amphibole asbestos.

**1477 SOLUBLE COMPONENTS OF ULTRAFINE PARTICULATE MATTER STIMULATE ENDOTHELIAL H2O2 PRODUCTION.**

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A growing body of evidence shows a strong association between particulate matter (PM) exposure and adverse cardiovascular health effects such as atherosclerosis and myocardial ischemia. The mechanisms by which PM cause cardiovascular dysfunction is unknown, but there is increasing evidence for a role of ultrafine (UF) particles with a diameter of <0.1 μm in cardiovascular effects of air pollution. While it is unlikely that PM can enter the circulation from the alveolar compartment, the soluble components of UF particles could directly cause oxidative stress, pro-inflammatory, and pro-coagulant changes in endothelial and cardiac cells. We hypothesize that the soluble components of ultrafine air pollution particles induce ROS formation in endothelial cells by activation of specific enzymatic sources. We quantified cellular H2O2 production using the Amplex Red assay in an endothelial cell line immediately following exposure to either UF particles, or their insoluble and soluble fractions at 10, 50, and 100 μg/ml. To identify cellular sources of ROS, we measured H2O2 production after cells were pretreated with chemical inhibitors of key endothelial ROS generating systems. We determined that the soluble components of UF particles are primarily responsible for increased H2O2 production. We also found that PM-induced H2O2 production was dose-dependent, and was inhibited by the NADPH Oxidase (NOX) inhibitor diphenylidionium (DPI). Mitochondrial and xanthine oxidase inhibitors (allopurinol, potassium cyanide, and rotenone) did not diminish particle-induced H2O2 production. Protein and mRNA analysis showed that NOX-4 was the primary NOX homologue present in this endothelial cell line. These data strongly suggests that NOX-4 is rapidly activated by the soluble components of UF particles to produce H2O2 in endothelial cells. This work has important implications for mechanisms of vascular effects of inhaled PM.

**1478 THE EFFECTS OF CONCENTRATED AMBIENT PARTICLES (CAPS) AND NICKEL NANOPARTICLES ON ENDOTHELIAL PROGENITOR CELL NUMBER AND FUNCTION.**

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Introduction: Particulate matter (PM), specifically nickel found on or in PM, has been associated with an increased risk of mortality in human population studies and significant increases in vascular inflammation, generation of reactive oxygen species (ROS), altered vasomotor tone, and potentiated atherosclerosis in murine exposures. Endothelial progenitor cells (EPCs), endogenous semi-pluripotent stem cells that aid in endothelial repair, are decreased in count and impaired in function for various chronic disease states. Thus, to determine if concentrated ambient particles (CAPs) and nickel nanoparticles can adversely affect the function and count of EPCs, various inhalation exposures were conducted. Methods: Several studies involving inhaled CAPs and nickel nanoparticle exposures were performed in order to quantify EPC counts using flow cytometry from lysed blood, bone marrow, or both, in ApoE -/- and C57BL/6 mice. Functional assessments were carried out on the nickel nanoparticle exposed mice and included a tube formation assay using bone marrow EPCs. Results and Conclusions: Significant differences between exposure and control groups for long term CAPs exposures and short term nickel nanoparticle exposures were observed. It appears that EPCs in bone marrow initially decrease, then rebound during the acute phase of the exposure (up to 4 months), and ultimately become depleted after sustained injury (6 months). EPCs in lysed blood remain constantly depressed over controls indicating that they may be quickly recruited from the circulation to sites of injury. Differences in tube formation were observed for the nickel nanoparticle exposed mice. In conclusion, this study shows that chronic inhalation of CAPs results in depressed EPCs in both bone marrow and lysed blood. Short-term nickel nanoparticle exposures also showed significant differences between groups, indicating that nickel may play an important role in EPC count and function.

**1479 INHALED TOLERANCE AND COMPARATIVE PK PROFILE OF INHALED NANOGENTM IN NON-HUMAN PRIMATES.**

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Engineered inhaled dry powder formulations of antibiotics have the potential to increase deposition and antimicrobial activity within the lung. As part of a larger program to test NanoGENTM, a jet-milled nanolactose formulation of gentamicin, for use as a post exposure prophylactic and treatment of Y. pestis and Tularemia, the inhaled maximum tolerated dose and PK profiles were determined in 3 African Green monkeys. A 3-way crossover PK design with matched clinical intravenous administration (IV), inhalation head dome (HD) and inhalation bolus delivery (BD) exposures was performed. An additional radioioabeled NanoGENTM imaging arm determined empirical dosing in the bd system. Mean particle size was acceptable in all formats, ~3.5 microns MMAD. Target doses were 0.3 (BD, HD, IV), 1(HD, IV) and 3(BD, HD). 1mg/kg. Max plasma levels were 1200(HD and IV) and 400 mg/ml (BD). Tmax was immediate (IV, BD) and delayed to 30 min post exposure (HD). Plasma AUC was HD>IV-BD. BD gamma scintigraphy of radioioabeled NanoGENTM indicated that ~37% of the total ejected material was delivered to the lung. BD NanoGENTM appeared to be retained in lung compared to IV and HD delivery, a potential indicator for enhanced antibacterial activity. The HD maximum exposure was 45 min at target aerosol concentrations of 1.6 mg/L. The equivalent maximum tolerated dose was 3 mg/kg active gentamicin or 5.4 mg/kg NanoGENTM. These data will be used to set doses for repeat dose toxicology studies as well as Y. pestis and Tularemia challenge studies. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Disease, National Institutes of Health (NIAID) and the Biomedical Advanced Research and Development Authority (BARDA), Department of Health and Human Services (DHHS), under Contract No. HHSN27220070030C.
As part of a larger program to test NanoGENT™ (a cryo-jet-milled formulation of gentamicin) for inhalation use in post exposure prophylaxis and treatment of P. aeruginosa, we determined the maximum tolerated dose (MTD) after a single nose-only inhalation exposure in male Sprague Dawley rats and male BALB/c mice. Six groups of 10 Sprague Dawley rats were exposed to NanoGENT™ aerosols (3 mg/l) or clean air for 8, 38, 78, 155, 180 or 0 minutes. Five rats per group were sacrificed 18 hours and 14 days post exposure. Three groups of 5 BALB/c mice were exposed to NanoGENT™ aerosols for 0 or 110 minutes to match the high dose of the rat exposures (10% deposition assumed) and sacrificed 18 hours or 14 days post exposure. NanoGENT™ average exposure concentrations during each of the 180 minute (rat) and 110 minute (mouse) exposures were 2.87 and 2.89 mg/l, respectively. Particle size [MMD (GSD)] during the rat and mouse exposures were 3.76(1.97) and 3.59(2.00) μm, respectively. Estimated inhaled deposited doses for rats were 1.44, 6.91, 13.34, 28.20, and 32.75 mg/kg. Estimated inhaled deposited doses for the mice were 33.14 mg/kg. No adverse signs or clinical observations were made in the rat groups. The BALB/c mice revealed white and red discoloration in the lungs in most rats in the 28.20 and 32.75 mg/kg/14 day necropsy groups corresponding to multifocal chronic interstitial inflammation with the most damage observed in the right cranial lung lobes. Based on the observed toxicity, the maximum tolerated dose was 100 μg/kg in both species. A no observed effect level (NOEL) or no observed adverse effect level (NOAEL) was not established in this study. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Disease, National Institutes of Health (NIAID) and the Biomedical Advanced Research and Development Authority (BARDA), Department of Health and Human Services (DHHS), under Contract No. HHSH27220070036C.
Polyvinylchloride (PVC) products frequently contain phthalates, a ubiquitous class of chemicals used as plasticizers. The most commonly used phthalate, Di-2-ethylhexyl phthalate (DEHP) is readily converted in the intestines to mono-2-ethylhexyl phthalate (MEHP). MEHP has been recognized as a testicular toxicant. Some studies have also implicated high dose adult exposures of MEHP as having adverse effects on the ovary in rats. However, few studies have addressed the adult effects of an in utero phthalate exposure model on the female. In this study, pregnant C57BL/6 mice were exposed via oral gavage to either 500 or 1000 mg/kg MEHP or corn oil vehicle at gestational days 17, 18, and 19. Offspring were then aged to early adulthood (approximately postnatal day 36) before sacrifice, while being monitored for alterations in estrous cycle. Body weights were similar between vehicle- and MEHP-exposed females (mean body weight of corn oil treated = 19.0 +/- 0.5; mean body weight of 1000 mg/kg = 16.8 +/- 1.6). The dates of vaginal opening, a common marker of sexual maturity, were also similar between treated and control mice (mean date of vaginal opening in corn oil treated = 29.85 +/- 1.3 days; mean date of vaginal opening in 1000 mg/kg at 30.75 +/- 2.20 days, respectively). Vaginal weights were not statistically different with mean combined ovary weights of 11.3 +/- 0.8 in control and 12.4 +/- 0.8 in 1000 mg/kg group. In utero exposed female offspring had a delayed onset of estrus in both the 500 and 1000 mg/kg MEHP treated groups relative to controls (Mean onset in control-treated animals at PND 35 +/- 1.9; mean onset in 1000 mg/kg and PND 39.8 +/- 1.6; 500 mg/kg PND 39. 9 +/- 3.7). Following onset of estrus, MEHP-exposed females displayed abnormal estrous cycles marked by a prolonged estrus stagen (Mean duration of estrus stage in controls, 1.44 +/- 0.53 days; mean estrus duration in 1000 mg/kg treated 2.9 +/- 0.64 days; mean duration in 500 mg/kg 2.80 +/- 0.86 days). These findings suggest that adult female mice exposed during an in utero window to MEHP show reproductive abnormalities.

Reproductive toxicity is one of the most complicated, time-consuming, and expensive endpoints to assess experimentally. At the moment there is no validated battery of alternative tests that would cover the different aspects of the reproductive cycle. The establishment of in vitro models for the evaluation of testsis development and testicular toxicity will provide important alternative approaches to in vivo systems and allow for new tools for the assessment of reproductive and developmental toxi-cants. Recently, we developed an in vitro three dimensional co-culture model of testis that contained an SF1 'consensus' sequence, was inhibited by DBP. Binding of the SF1 antibody, in a region of the Cyp11a promoter <200bp from PPAR-α binding could interfere with SF-1 binding in this region. Analysis of the sequences bound by these proteins were hybridized against non-enriched genomic or RNA polymerase II (RPOL2) antibodies. Labeled IP-DNA samples enriched for activated receptor sequestration. To test this hypothesis we have performed PITMD-gene-focused chromatin immunoprecipitation (ChIP) microarray analysis. Rats were exposed throughout gestation in utero to DBP (500mg/Kg, p.o. to dams) and gestational day (GD) 15 and 19 fetal testes were formaldehyde fixed and DTS were immunoprecipitated (IP) using SF1, CREB binding protein (CBP), PPARα or RNA polymerase II (RPOL2) antibodies. Labeled IP-DNA samples enriched for sequences bound by these proteins were hybridized against non-enriched genomic DNA on PITMD-ChIP microarrays containing probes spanning promoters of DBP 'target' genes. Comparison of binding events between control and DBP-treated GD19 tests showed that SF1-binding in a region of the Cyp11a promoter that contained an SF1 'consensus' sequence, was inhibited by DBP. Binding of the SF1 coactivator CBP was increased by DBP in this region. In GD15 and GD19 fetal testes DBP treatment increased binding, relative to control, of a protein recognized by the PPARα antibody, in a region of the Cyp11a promoter <200bp from the site bound by SF-1. Hence, these data suggest that DBP-induced alterations in PPARα binding could interfere with SF-1 binding in this region. Analysis of the data with regard to other PITMD genes is ongoing. This work was supported by ExxonMobil Petroleum & Chemical.


Testicular dysgenesis syndrome (TDS) has been clearly linked to environmental po-lutants exposure. The chemicals may act through disruption of steroid hormone signaling, but alterations of other signaling pathways are also believed to cause TDS (1). Phthalate esters, such as di-ethylhexyl phthalate (DEHP), are used in industry in the manufacture of plastics in order to improve their flexibility. Everyday life products such as lubricants, cosmetics, and paints may contain phthalates. Therefore, phthalates may be found in a number of environments, from streambeds to household dust and dairy products. Epidemiological and animal studies evaluating the impact of phthalates on the male reproductive tract provide evidence of degradation in sperm quality, of increase in the incidence of genital birth defects, and of testicular cancer (2, 3). Gland cell line-derived neurotrophic factor, or GDNF, is a growth factor involved in the self-renewal of spermatogonial stem cells (SSCs). GDNF is critical for continuous spermatogenesis, since the testis knock out model is not able to maintain the germ line. Impairment of stem cells self-renewal can induce both decreased sperm count and sperm quality, or may potentially lead to testis cancer. This work, using the SSC-based cell line C18-4 as a model, demonstrates that mono-ethylhexyl phthalate (MEHP, the main metabolite of DEHP) disrupts GDNF signaling. MTS-based cell proliferation assays revealed a decrease of C18-4 cell viability in a time and dose dependent manner when the cells were exposed to MEHP. Western blotting results showed a decrease of nearly 30% in Erk1/2 activation, a cellular effector of GDNF signaling. This effect is associated with a decrease of cFos but not NMyc protein expression, which are both transcription factors up-regulated by GDNF. Taken together, our data suggest that MEHP exposure triggers a disruption of GDNF signaling, leading to a decrease in spermatogonial stem cell proliferation.

Phthalates decrease spermatogonial stem cells proliferation by altering GDNF signaling.
1490 BRONCHOALVEOLITIS OLITRANTS-LIKE LESIONS IN RATS TREATED WITH DIACETYL, ACETIN, OR ACETYL PROPIONYL BY INTRATRACHEAL INSTILLATION.

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Diacetyl (DA) and acetoin (AC) are major volatile components of artificial butter flavoring (ABF) implicated in causing bronchiolitis obliterans (BO) in workers. Acetyl propionyl (AP) may be used as a substitute for DA in ABF. DA inhalation does not cause bronchiolar injury or BO in rodents possibly due in part to the protective effects of the upper respiratory tract (URT). Therefore, to circumvent the protective effect of the URT, rats were treated with DA, AC or AP by intratracheal instillation (IT). Male, Sprague-Dawley rats received a single dose of sterile water (vehicle), DA (125 mg/kg), AC (125 mg/kg) or AP (140 mg/kg) by IT on day 0. Bronchoalveolar lavage fluid (BALF) was evaluated on days 1, 3 and 7, and lungs were examined microscopically on days 1, 3, 7 and 14. Body weights of treated rats were similar to controls at all time points. AC and AP caused a transient increase in BALF cell numbers and protein at day 3. LDH activity was elevated at all time points in BALF of rats treated with AC or AP. DA did not cause changes in lung weights. Rats exposed to AC or AP demonstrated increased BALF cytokines. A number of cytokines were elevated in rats treated with DA, AC, and AP. All 3 ketones caused significant increases in MCP-1, MCP-3, SCF, and MPO in the day 7 BALF. Although differences were found between the 3 chemicals a general progression of histological changes occurred in the airways of treated rats. Day 1: inflammation, necrosis and denudation of bronchiolar epithelium. Day 3: epithelial regeneration and hyperplasia, and bronchial polyps of early type. Day 7: bronchiolar polypoid and intramural fibrosis. Day 14: regeneration of bronchiolar/bronchial epithelium sometimes with decreased incidences of endobronchial fibrosis. These results demonstrate that DA, AC, and AP can cause BO-like lesions in rats if sufficient chemical reaches the bronchioles by IT administration. The mechanism is likely a general repair response to caustic chemicals.

1492 INHALATION TOXICITY OF ACETYL PROPIONYL IN RATS AND MICE.

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Acetyl propionyl (AP; 2,3-pentanedione) is a diketone commonly used in the flavoring industry. AP is structurally similar to diacetyl (DA; 2,3-butanedione), a diketone used in artificial butter flavoring (ABF) and other flavorings. Inhaled DA is toxic to the upper respiratory tract of rodents and has been implicated in causing bronchiolitis obliterans (BO) in exposed workers. The potential use of AP as a substitute for DA in ABF is a concern because of the lack of toxicity data for AP, and its structural similarity to DA. Studies were conducted to provide inhalation toxicity data for inhaled AP in Wistar-Han rats and B6C3F1 mice. In an initial study, male rats and mice were exposed to 400 ppm AP for 6h/d. After 2 exposures 2/6 rats were found dead and 4/6 were sacrificed in moribund condition. After 4 exposures 2/6 mice were found dead and 4/6 were sacrificed in moribund condition. All exposed rats and mice exhibited marked, acute suppurative and necrotizing rhinitis, marked acute e erosive and necrotizing tracheitis, and most showed acute maxillary sinusitis. Necrosis of either the naso- or maxillo-turbinate bone was present in all exposed mice, and was absent in rats. All exposed rats and 2/4 mice showed areas of olfactory epithelial degeneration, primarily in the dorsal meatus of level 2. In a subsequent study, male and female rats and mice were exposed to 0, 50, 100, or 200 ppm AP 6h/d, 5d/w 2w. Early deaths of 4/6 male rats, 2/6 female rats, and 1/6 female mice occurred only at 200 ppm. All male mice survived. Body weights of rats and mice exposed to 200 ppm were significantly less than controls. Relative kidney and liver weights were increased in 200 ppm female mice. Absolute lung weights were increased in 200 ppm male rats and relative lung weights were increased in the 200 ppm male and female rats, and female mice. These results indicate that inhaled AP causes upper respiratory tract toxicity in rodents with features similar to that of DA.

1493 DIESEL EXHAUST PARTICULATE ALTHERS ENDOTHELIAL CELL SOS, ENDOTHELIN AND MCP1 GENE EXPRESSION IN TWO IN VITRO MODELS OF EXPOSURE.

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Diesel exhaust particulate (DEP) exposure influences blood pressure (BP) and vascular reactivity. While the mechanisms are mostly unknown, DEP exposure increases the expression of endothelin (EDn1) as well as endothelium and inducible nitric oxide synthases (eNOS, iNOSOS) in vivo. After inhalation of DEP, alveolar macrophages engulf particles and secrete proinflammatory cytokines, possibly leading to the systemic effects seen during in vivo exposure. An alternative hypothesis is that a fraction of DEP may pass through the pulmonary epithelium, enter the circulation, and directly contact the endothelium. Thus we investigated the effects of DEP on Edn1, Ece-1, eNOS, iNOS, MCP-1, GCCL and GCLM gene expression in two in vitro models: 1) Direct DEP addition to mouse endothelial (SVEC) cells; 2) DEP addition to mouse RAW macrophages in a co-culture system using transwell inserts whereby SVEC cells are exposed to secretions of the RAW cells but not DEP directly. In the direct exposure model, DEP increased eNOS, iNOS, MCP-1 and GCLC mRNA transcript levels, but decreased Edn1 mRNA transcript levels. In the co-culture system, DEP exposure increased cytokine expression in the RAW compartment; perfusion occurs only in the submucosa. Model predictions were compared to values for average uptake efficiencies obtained from published human exposure studies. Model output agreed closely with these data, validating the model. The model was then used to examine inhalation dosimetry of diacetyl (2,3-butanedione), a highly soluble vapor (bloodair partition coefficient 550) associated with the development of bronchiolitis obliterans in some occupational settings. Under quiet mouth breathing (9 l/min, 1 ppm), the model estimates 25% and 45% of inspired diacetyl penetrates to the bronchioles with and without inclusion of respiratory tract metabolism, respectively. During exercise (25 l/min), the mass of diacetyl delivered to bronchioles increases -four-fold (irrespective of the inclusion of metabolism). These results suggest significant amounts of inspired diacetyl penetrate to the bronchioles of the human, with greater penetration occurring during exercise than rest.
Naphthalene and styrene are sensory irritants in the mouse but little is known about the mechanism(s) through which this response is initiated. These two agents are also nasal cytotoxicants; the cytotoxic response is thought to be due to the formation of epoxide metabolites within the nose by cytochrome P450 (CYP) metabolism. Both agents induce changes in the expression of genes important in the synthesis of vasoactive molecules in the endothelium when either directly exposed to DEP or when exposed to the secretions of macrophages exposed to DEP. This work was supported by NIEHS grants 1P50ES015915, P30ES07073 and T32ES070732.

**1494 CYP-DEPENDENT SENSORY IRRITATION OF NAPHTHALENE AND STYRENE.**

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**1495 AUTOMATED AEROSOLIZATION, DISPERSION, AND CONCENTRATION CONTROL OF SILICA POWDER FOR USE IN INHALATION EXPOSURE STUDIES.**

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Inhalation exposure systems are necessary tools for determining the dose response relationship of inhaled toxicants under a variety of exposure conditions. The objective of this project was to develop an automated computer controlled system to expose small laboratory animals to precise concentrations of uniformly dispersed airborne silica particles. An acoustical aerosol generator was developed which was capable of suspending a respirable fraction of particles from bulk powder. The aerosolized silica output from the generator was introduced into the throat of a venturi tube. The high velocity air stream within the venturi tube further broke up and dispersed the aerosolized powder. This air was then used to expose small laboratory animals to constant aerosol concentrations, up to 20 mg/m3, for durations lasting as long as 8 hours. The number of viable particles and morphological features of the silica aerosol delivered to the exposure chamber were measured to verify that a fully dispersed and respirable aerosol was being delivered to the animals’ breathing space. The inhalation exposure system utilized a combination of air flow controllers, particle monitors, data acquisition devices, and custom software with automatic feedback control to achieve constant and repeatable Exposure chamber temperature, relative humidity, pressure, aerosol mass concentration, and particle size distribution. The automatic control algorithm was capable of delivering median aerosol concentrations to within +/- 0.2 mg/m3 of a user selected target value during inhalation exposures lasting from 4 to 8 hours. The system was capable of reaching 99% of the target value in less than 15 minutes during the start up phase of an inhalation exposure. This exposure system provides a highly automated tool for exposing small laboratory animals to precise concentrations of uniformly dispersed airborne silica particles.

**1496 COMPARISON OF COMPOSITION OF PM2.5 FROM PAVED ROADS AND IN AMBIENT AIR.**

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A portion of exposures to fine respirable particulate matter (PM2.5) occurs on or near paved roads. Much attention has been given to vehicle tailpipe emissions, but little is known about the composition of fine (PM2.5) paved road dust (PRD) re-suspended by traffic. PRD was collected by air re-suspension from active traffic surfaces of residential and urban arterial streets, freeways, center-city street canyons, and industrial areas in Atlanta, El Paso, Los Angeles, and New York City. The PRD was sieved and re-aerosolized, and the PM2.5 fraction was collected and analyzed chemically. Results were compared to the composition of ambient airborne PM2.5 at nearby speciation monitoring stations. Although the samples were not matched perfectly in location and time, the comparison is revealing. As expected, the PRD contained a much larger crustal component and a smaller inorganic component than ambient PM2.5. An interesting finding was that PRD had a much larger component of reactive metals (chromium, copper, iron, nickel, vanadium). The results suggested that the inhalated “dose” of reactive metals during short times on or adjacent to roadways may be equivalent to the dose received from many hours of exposure to regional ambient PM2.5. Research funded by multiple government and industry sponsors through the National Environmental Research Center.

**1497 PRELIMINARY RESULTS INVESTIGATING DIESEL EXHAUST PARTICULATE MEDIATED LUNG INFLAMMATION IN WILD TYPE AND GCLM-HETEROZYGOUS MICE.**

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Diesel exhaust particulate (DEP) is a major component of airborne particulate matter (PM) and exposure to DEP has been associated with pulmonary irritation, inflammation and exacerbation of asthma symptoms. DEP and PM can produce an inflammatory response in the lungs by producing reactive oxygen and nitrogen species. The induction of oxidative/nitrosative stress can lead to the synthesis of proinflammatory cytokines, attracting neutrophils to the site of injury. Glutathione (GSH) is an important antioxidant tri-peptide thiol composed of glutamate, cysteine and glycine. The rate-limiting enzyme in the synthesis of GSH is glutamate cysteine ligase (Gcl), composed of catalytic (Gclc) and modifier (Gclm) subunits. Our lab has developed Gclm null mice that may be useful for investigating the role of oxidative stress and GSH in DEP induced pathology. Thus, we exposed C57Bl/6 wild type (WT) and Gclm heterozygous (HT) mice to DEP via intranasal instillation. Animals received a total of 20μl of PBS or 20μl of a 10 mg/ml DEP solution (10 μl per nostril). After 6 hours, mice were sacrificed and bronchial alveolar lavage (BAL) was performed. BAL cells were stained with the macrophage marker F4/80 and the granulocyte marker Gr1+, and evaluated via FACS for the percentage of F4/80+ and Gr1+ cells. DEP instillation induced neutrophilia in both WT and HT mice and preliminary data indicate that HT mice are slightly more sensitive than WT mice to this effect. These data suggest that GSH levels may influence the degree of DEP-induced inflammation in the lung. This work was supported by NIEHS grants 1P50ES015915, P30ES07073 and T32ES070732.

**1498 DIFFERENTIAL ELECTROCARDIOGRAM EFFECTS IN NORMAL AND HYPERTENSIVE RATS AFTER INHALATION EXPOSURE TO TRANSITION METAL RICH PARTICULATE MATTER.**

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Inhalation of particulate matter (PM) associated with air pollution causes adverse effects on cardiac function including heightened associations with ischemic heart disease, dysrhythmias, heart failure, and cardiac arrest. Some of these effects have been attributable to transition metal components of PM. Recent epidemiologic data (Bell et al, 2009) shows associations of cardiac hospitalization with fine PM-associated Ni and V. Residual oil fly ash (ROFA), a waste product of fossil fuel combustion from boilers, is rich in the transition metals Fe, Ni, and V, and when released as a fugitive particle, is an important contributor to ambient fine particulate air pollution. We hypothesized that a single acute inhalation exposure to transition metal-rich particulate matter designed to mimic ROFA will cause greater cardiopulmonary toxicity in Spontaneously Hypertensive (SH) rats than in similarly suspended Wistar Kyoto rats with normal blood pressure. Rats were exposed once by nose-only inhalation for 4 hours to approximately 500 μg/m3 of a synthetic particulate matter consisting of Fe, Ni and V sulfates that is similar in composition to a well-studied ROFA sample. PM exposure in SH rats caused an increase in T-wave
1499 A TWO-WEEK (9-EXPOSURE) INHALATION STUDY IN F344 RATS: PULMONARY TOXICITY OF FOUR UCON™ FLUIDS.

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UCON™ Fluids and Lubricants are polyalkylene glycol-based synthetic products used in a variety of industrial fluid applications. The UCON™ Fluid product line contains water-soluble 50-HB (terminal propylene oxide:propylene oxide) and water insoluble LB (terminal propylene oxide) lubricants. The 50-HB fluids, at exposures ≤100 mg/m³, produce lung toxicity that increases in severity with increasing molecular weight. The highest molecular weight product in the family, 50-HB-5100, increased lung weights and produced histopathological changes in the lung at exposures as low as 5 mg/m³. The present study investigated the effect of LB-285, LB-625, and LB-1715, (larger number-higher molecular weight), to cause lung toxicity in Fischer 344 rats compared to 50-HB-5100. Groups of 10 male rats were exposed to 99, 99, 101, and 25 mg/m³ of LB-285, LB-625, LB-1715, and 50-B-5100, respectively; 6 hours/day, 5-days on, 2-days off, 4-days on for 9 exposures in 11 days in whole-body exposure chambers. The mean particle size of the test aerosols was 1.3, 1.5, 1.5, and 2.3 μm, respectively. All animals were observed twice daily for mortality and morbidity; clinical signs of toxicity and body weights were recorded. All animals were necropsied; selected tissues were collected, weighed, fixed, and processed for histopathological examination. No mortality or test article-related clinical signs occurred in any UCON™ fluid-exposed group. Body weight gains were reduced for all measured intervals (-12% to -40%) in all UCON™ fluid-exposed groups; however, mean body weights were reduced only -2% to -8%. Consistent with previous studies, 50-HB-5100 induced lung effects: increased lung weights with histiocyte infiltrate and alveolitis. No exposure-related lung effects were observed in LB-exposed groups. The basis for the observed difference in pulmonary toxicity of the UCON™ fluids is not known, but may reflect differences in pulmonary absorption due to the greater water solubility (1000x) and surfactant properties of 50-HB-family compared to LB-family products.

1500 OXIDIZED LIPIDS AND LIPID MEDIATORS ARE INVOLVED IN CARDIOVASCULAR INJURY INDUCED BY DIESEL EXHAUST PARTICLES (DEP) AND OZONE.

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The mechanisms by which air pollutants induce cardiac and vascular injuries are unknown. We hypothesized that these injuries involve alterations in aortic membrane and lipid mediators. We exposed male Wistar Kyoto rats (12-15 wk old), nose-only to air, ozone (O3; 0.5 ppm), bulk DEP(2.0 mg/m³), or DEP+O3, 5 h/d, 1 d/wk for 16 wk. In an acute study, rats were exposed to air, O3 (0.5 or 1.0 ppm), or DEP (2.0 mg/m³) for 5 h/d x 2 d. We analyzed gene and protein expressions of oxidized LDL receptor 1 (LOX-1), and markers of injury in the aorta, lung and heart. Aortic and cardiac phospholipid-fatty acids and conjugated dienes (lipid peroxidation) were analyzed by gas chromatography-mass spectrometry in the acute study. Cardiac mitochondrial fatty acids were analyzed in the subchronic study. Modest pulmonary inflammation was noted in acute and subchronic O3 and/or DEP exposed rats. Gene expression in the lung and heart did not change with any conditions in the subchronic study. However, marked induction of LOX-1 mRNA and protein (O3+DEP-O3+DEP) was observed in the aorta. This was accompanied by induction of aortic markers of thrombosis, proteases and endothelin. The alterations in aorta were similar in rats exposed to O3 as compared to DEP; and were additive after the subchronic exposure. Conjugated dienes in the aorta were elevated in rats acutely exposed to O3 but not DEP. Loss of phospholipid-fatty acids was noted in the mitochondrial fraction of hearts in rats exposed to O3 or DEP. These results demonstrate a potential role of membrane lipid oxidation and LOX-1-mediated signaling in vascular oxidative and inflammatory injury following O3 and DEP exposure. (Does not reflect U.S. EPA policy). Supported in part by EPA/UNC #CTB29471.

1501 PARTICULATE INHALATION IN RATS CAUSES CONCENTRATION-DEPENDENT ELECTROCARDIOGRAPHIC, AUTONOMIC, AND CARDIAC MICRONOMA EXPRESSION CHANGES.

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Recently, investigators in key epidemiologic studies demonstrated associations between exposure to fine particulate matter (PM)-associated metals and increased hospital admissions (Ni and V) and cardiovascular mortality (Ni and Fe). Residual oil fly ash (ROFA), a waste product of fossil fuel combustion from boilers, is rich in the transition metals Ni, V, and Fe, and when released as a fugitive particle, is an important contributor to ambient air fine PM. We hypothesized that a single inhalation exposure to metal-rich PM will cause concentration-dependent cardiopulmonary toxicity in Spontaneously Hypertensive (SH) rats. Rats were exposed once by nose-only inhalation for 4 hours to 3.5 mg/m³, 1.0 mg/m³ or 0.45 mg/m³ of a synthetic PM (dried salt solution) consisting of Fe, Ni and V sulfates that is similar in composition to a well-studied ROFA. High PM exposure caused several electrocardiographic and autonomic alterations during and immediately after exposure including decreased T-wave amplitude and area, ST segment depression, reduced HR, decreased LF/ HF (a marker of heart rate variability) and increased non-conducted P-wave arrhythmias. High PM exposure also caused pronounced pulmonary inflammation and hyper-responsiveness and down-regulation of the expression of 24 microRNAs (miRNA) in the myocardium one day after exposure. The low and middle concentrations decreased HR, but had no effects on the ST segment, arrhythmias, pulmonary inflammation or hyper-responsiveness, and affected expression of fewer miRNAs. The low concentration did increase T-wave amplitude and area while the middle concentration increased QRS area and decreased LF/HF. Taken together, the data suggest that PM concentration influences both the severity of cardiotoxicity and the relative contribution of specific mechanisms in the mediation of adverse effects (This abstract does not reflect EPA policy).

1502 MAST CELLS CONTRIBUTE TO ALTERATIONS IN VASCULAR REACTIVITY AND EXACERBATION OF ISCHEMIA/REPERFUSION INJURY FOLLOWING ULTRAFINE PM EXPOSURE.

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Increased ambient fine particulate matter (FPM) concentrations are associated with increased risk for short- and long-term adverse cardiovascular events. Ultrapure PM (UFPM) due to its size and increased surface area may be particularly toxic. Mast cells are recognized for their role in allergy, asthma and cardiovascular disease, yet their role in cellular response to PM is unknown. The aim of this study was to examine activation of mast cells in the pulmonary and cardiovascular pathology following exposure to UFPM. Mouse bone marrow-derived mast cells (BMMC) were exposed in vitro to UFPM and both IgE and non-IgE mediated mast cell activation was examined. Additionally, we assessed lung inflammation, aortic vascular reactivity and myocardial ischemia/reperfusion (IR) injury in UFPM instilled C57Bl/6 and B6.Cg-Ki/Kit-w+ mouse cell deficient mice. Exposure to UFPM (0-100 μg/ml) did not significantly affect BMMC degranulation, however, UFPM promoted inflammatory cytokine production by BMMCs. In vivo studies revealed that mice exposed to UFPM (100 μg, n=6/group) exhibited increased pulmonary inflammation. Further, UFPM instilled C57Bl/6 mice displayed increased myocardial IR infarct size by ~40% as compared to saline controls, while B6.Cg-Ki/Kit-w+ mice displayed only a ~10% expansion. Lastly, C57Bl/6 mice exposed to UFPM exhibited decreased aortic vascular constriction to norepinephrine that was largely absent in
Workers producing microwave popcorn are at increased risk for severe, fixed airways obstruction. Human disease correlates with exposure to diacetyl (2,3-butanedione), a 4-carbon, α,β-diketone component of butter itself and many butter flavorings. In rats, acute diacetyl inhalation damages epithelium in nose, trachea and large intrapulmonary airways, with the greatest damage in nose, an injury distribution explained in part by the pharmacokinetics of inhaled diacetyl. A 5-carbon α,β-diketone, 2,3-pentanedione, is also used as a flavoring. The acute respiratory toxicity of 2,3-pentanedione was investigated in this study because of structural similarities to diacetyl. Male, Sprague-Dawley rats inhaled 0, 118, 241, 318 or 354 ppm 2,3-pentanedione for 6 hr, were sacrificed the next day, and nose, trachea, and lung were assessed by histopathology. Airway epithelial changes included degeneration, apoptosis, necrosis and neutrophilic inflammation, with nasal epithelium being most affected. As exposure concentration increased, epithelial damage and inflammation increased in severity and extended deeper into the respiratory tract, with necrosuppurative tracheitis present in all rats inhaling 354 ppm. Physical examinations suggested delayed onset of toxicity. To investigate potential delayed toxicity, additional rats were exposed to 318 ppm, 2,3-pentanedione and sacrificed immediately (<2 hr) or 1 day (18 – 20 hr) after exposure. In the 1st nasal section (T1), minimal to mild, epithelial cell degeneration, apoptosis and individual cell necrosis observed immediately after exposure progressed with time post-exposure, developing into moderate to marked, multifocal and coalescent, necrosuppurative rhinitis. In the 2nd nasal section (T2), similar to diacetyl, injuries airway epithelium in rats, predominately in nose, but also affects deeper airways. In addition, clinical and histopathologic toxicity are delayed after 2,3-pentanedione inhalation.

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Background: Life expectancy may start to decrease in developed countries for the first time in recent history due to obesity. Our recent study demonstrated that fine particulate matter (diameter <2.5 μm, PM2.5) exposure increased adipocyte size in adult C57BL/6 mice fed high fat chow (HFC). We then hypothesized that PM2.5 exposure also led to increased proinflammatory genes TNF-α1, IL-1β, IL-6, and MCP-1) by ELISA, elevated macrophage infiltration (by electron paramagnetic resonance) in visceral adipose tissue, and macrophage M1/M2 gene changes that may be mediated by NADPH oxidase subunit p47phox-/- mice. These findings have significant public health impact on both children and adult health.

Methods: Mice at week 3, fed normal chow (13% calories from fat) or HFC (60% calories from fat), were randomly exposed to PM2.5 or filtered air in a whole-body exposure system for 10 weeks. Results: Glucose tolerance tests indicated glucose metabolic abnormality and IR in PM2.5-exposed mice, which exhibited increased constriction to phenylephrine and decreased relaxation to acetylcholine and insulin by myograph, increased adherent and rolling monocytes in the cremaster microcirculation by intravital microscopy, increased visceral fat mass by MRI, enhanced systemic inflammation (TNF-α, IL-6, and MCP-1) by ELISA, elevated macrophage infiltration (by IHC) and superoxide anion generation (by electron paramagnetic resonance) in visceral adipose tissue. Boyden chamber assay showed that PM2.5 exposure increased visceral fat chemotaxis to monocytes than subcutaneous or perivascular fat. PM2.5 exposure also led to increased proinflammatory genes TNF-α, NOS2, and IL-6 (M1, classical activated) and decreased IL-10 (M2, alternative activated). These adipogenic and pro-inflammatory effects were exaggerated by HFC feeding but significantly reduced in p47phox-/- mice. Conclusion: Exposure to PM2.5 in childhood potentiates T2DM, systemic IR, inflammation in visceral adipose tissue, and macrophage M1/M2 gene changes that may be mediated by NADPH oxidase subunit p47phox-/. These findings have significant public health impact on both children and adult health.

Health effects associated with PM show differences depending on particle size, season, and location. We hypothesized that the differences in the PM composition account for these varied effects. Nearly 400 size-fractioned PM samples were collected from 5 U.S. and 2 Chinese cities in 2007-08. A high volume cascade impactor was used to collect samples and ICP-MS was used to determine elemental composition. Human pulmonary microvascular endothelial cells (HPMEC-ST1.6R) and bronchial epithelial cells (BEAS-2B) were exposed to PM for up to 24 hr. As measured by LDH release, no significant toxicity was measured for any dose (10, 50, and 300 μg/m3) in either cell line. Results showed that season strongly influenced reactive oxygen species (ROS) formation (as measured by oxidation of a fluorescent dye) for some but not all cities. For example, winter Ann Arbor samples caused more ROS formation than summer samples from China. City-dependent differences were also observed in PM size fractions collected in China. Beijing PM, collected during the 2008 Olympics, caused a greater production of ROS than Tianjin (130 km from Beijing) PM collected either during or after the Olympics. This suggests that source control measures instituted before and during the Olympics may not have sufficiently reduced important PM components. Our results support the hypothesis that the elemental composition of PM drives health effects. Future studies should correlate in vitro findings with individual elemental data and source apportionment. In addition, evaluation of gene expression will provide insight into the possible cellular mechanisms for PM-induced cardiopulmonary effects.
Epidemiological studies show a positive correlation between traffic-related air pollution and increased rates of cardiovascular morbidity and mortality; however, the underlying mechanisms are still unknown. We have previously reported that vascular oxidative stress levels are significantly elevated in atherosclerotic apolipoprotein K0 (Apoe−/−) mice exposed to vehicular emissions (VE), resulting in increased oxidized LDL (oxLDL) and expression of its receptor lectin-like-ox-LDL receptor (LOX-1), as well as increases in endothelin-1 (ET-1) and matrix metalloproteinases (MMPs). HMG-CoA reductase inhibitor (statin)-treatment has been shown to effectively reduce oxLDL, thus to test the hypothesis that VE-induced oxLDL is mediated by VE-receptor expression and activity, as well as increased MMP-9, likely mediated through induction of the LOX-1 receptor. Funded by NIH R09EO15656(ALK)& NIH 1DE5014639(MJC).

1512 EXACERBATED INFLAMMATORY LUNG INJURY IN NEPRILYSIN NULL MICE FOLLOWING DIESEL PARTICLES EXPOSURE.

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Neprilysin (NEP) is a key cell surface peptidase and plays an important role in the development of pulmonary disorders including asthma, COPD, and lung cancer. In this study, to simultaneously characterize the dose-response of diesel exhaust particles (DEP) and the role of NEP in pulmonary response, we conducted a 2 X 3 factorial design [54 mice = 9 mice/group X 6 groups, NEP wild-type vs. null (NEP−/−) mice that were exposed to control (0), low (10 µg), high doses (100 µg) of DEP (SRM 2975), respectively]. At 7 days following DEP or PBS (control) instillation, bronchoalveolar lavage (BAL) fluid and lung tissue were collected. The results showed that total cell number in BAL increased approximately 1.7- and 2.4-fold in wild-type mice, while approximately 2.3- and 3.7-fold in NEP−/− mice after the low- and high DEP treatment, respectively. IL-6 increased in a dose-dependent manner in wild-type mice or NEP−/− mice. NEP−/− mice displayed much larger increase for IL-6 than wild-type mice after DEP treatments. IL-1β and IL-10 in NEP−/− mice, but not in wild-type mice, were significantly increased following the high dose of DEP instillation. Histopathological examination showed that DEP-induced tissue injury in NEP−/− mice was obviously worse than that of wild-type mice, as indicated by airway and alveolar interstitial edema. Although the mechanism remains to be determined, lack of NEP could cause the increased susceptibility to injury or exacerbate the inflammatory responses of mice to DEP treatment by allowing higher releases of specific cytokines from lungs. This finding suggests that loss of NEP may be a factor that is mechanistically linked to inflammatory injury or airway disease susceptibility (Supported by H1).
ppm after 12 min and persisted until the end of the exposure period. Subsequently, viability was measured and compared to cultures exposed to filtered air only. After a 24 h recovery period from exposure the viability was 91% and it was 80% 48 h post-exposure. These experiments were conducted to establish dilution ratios of smoke in air leading to cytotoxic effects. Reduced glutathione (GSH) was increased 1.6 fold after 24 h in H322 by 2R4F total particulate matter (TPM, 5mg/l). Viability of cells was 96% under these conditions. The expression of the DNA repair protein poly (ADP-ribose) polymerase-1 (PARP-1) was induced by 1.4 fold compared to the control group with TPM (0.05mg/l) for 24 h. These data indicate that substantial stress response occurs in lung cells well below cytotoxic concentrations of TPM already within 24 and 48 h. Further steps are to adapt the system for the needs of repeated exposure of primary human lung cells from explants and to handle small size particles.

Unhealthy levels of particulate air pollution affect 30% of the US population. Epidemiologic studies associate particulate matter (PM) exposure with increased respiratory disease in children. Phase I and II enzymes, both key to activation and detoxification of polycyclic aromatic hydrocarbons (PAH) within PM, differentiates postnatal in the lung. PM contains organic compounds (including PAH) at various levels depending on source. In this study, we exposed 7 day old postnatal and adult rats exposed to PM of two different content (PM) and low organic content PM (DFP). Rats were exposed for a 6 hour period to 2x10^7 particles of either type and the lungs were analyzed at 24 and 48 hrs post exposure. Protein expression was determined using immunohistochemistry and airway associated gene expression was determined by quantitative real time RT-PCR on microdissected airways. There were substantial differences in airway expression of key enzymes between postnatal and adult rats. Adult had 300 fold greater expression of CYP1A1, 2 fold greater expression of glutamine cysteine ligase (GCL), a key enzyme in glutathione synthesis, yet antioxidant heme oxygenase-1 remained unchanged within the airways. FFP exposure increased CYP1B1 in both postnatal and adult rats, but the temporal pattern differed by age. FFP caused a 10 fold increase in CYP1A1 gene expression in postnatal rats only, adults was unchanged. In contrast, DFP exposure did not change CYP1A1 airway expression in either age. Both the antioxidant Nrf2 transcription factor and GCL increased de-spite relatively low PAH content in the DFP. We conclude that: 1) basal levels of many key cytochrome P450s are lower in postnatal rats, 2) that the temporal pattern and expression of metabolic enzymes vary depending on the organic carbon content of the exposure and the age of the animal. Funded by: NIH ES067600 and U.S. EPA RD-83241401.

Increased ambient particulate matter (PM) exposure in urban areas has been associated with increased mortality; yet, little is known about health effects from PM around dairy farms. Our lab has observed significant inflammation as seen with neutrophil (PMN) recruitment to the lung during dairy barn PM exposure, and it is important to know the impact of PMNs as they can either reduce injury after a PM exposure or add to it. We hypothesized that PMN recruitment to the mouse lung reduces cytotoxicity after PM exposure. Male C57Bl/6 mice received H2O or add to it. We hypothesized that PMN recruitment to the mouse lung reduces cytotoxicity after PM exposure. Male C57Bl/6 mice received one of four treatments: intratracheal injection (i.p.) with histidine (H), a PMN-depleting drug, and IT with dairy barn PM with a mass median aerodynamic diameter of 2.5-10μm (PM10); IT with PM10 alone; i.p. with HU alone; and IT with the saline vehicle. Bronchoalveolar lavage fluid (BALF) and blood plasma samples were collected to determine the extent of PMN recruitment and cytotoxicity. PMN recruitment was estimated via BALF cell counts, and the extent of cytotoxicity was determined by lactate dehydrogenase (LDH) and bichrocinnic acid assays. There was a significant increase in PMNs recruited to the lung in both the HU/PM10 group and the PM10 alone group as compared to the HU alone and PBS control groups. However, the HU/PM10 group had a 50% reduction in recruited PMNs versus the PM10 alone group. Trends with the LDH assay show a high organic content in cytotoxicity with the HU/PM10 group as compared to the PM10 alone group. These experiments suggest that depletion of PMNs is associated with less cytotoxicity. Furthermore, this suggests that PMNs contribute to pulmonary injury after dairy barn PM10 exposure.

**1516 INFLAMMATORY RESPONSE TO INHALED LPS IN NON-HUMAN PRIMATES:TREATMENT WITH STEROIDS OR CARBON MONOXIDE.**

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The objective of this study was to determine if inhaled carbon monoxide (CO) gas is efficacious in reducing lipopolysaccharide (LPS) induced lung inflammation in cynomolgus macaques. Pretreatment with a glucocorticoid steroid, budesonide, was used as a positive control in these studies in order to confirm the CO cytoprotective effects in the context of currently used therapies. From previous work, CO appears to have a therapeutic use at low levels. However, the remarkable protection of CO observed in previous rodent studies must be validated in a species that is more similar to humans. Before CO can be identified as a therapeutic agent for preventing or treating diseases associated with pulmonary inflammation in humans. Initial tests with LPS inhalation in cynomolgus macaques show profound influx of neutrophils and moderate increases in lymphocytes in the airway. These changes in resident cell populations were back to baseline within 2 weeks of exposure. Analysis of cytokine release in lavage fluid shows that CO exposures decreased tumor necrosis factor alpha (TNFα) release following LPS induction of pulmonary neutrophilia. CO alone was able to decrease TNFα expression below homeostatic levels. CO did not appear to affect IL-6 or IL-8 release. Pretreatment with budesonide prior to LPS exposure in primates caused decreased expression of TNFα, IL-6 and IL-8. While budesonide pretreatment reduced LPS induced neutrophilia by approximately 84%, CO treatment post LPS exposure was able to reduce the inflammation by approximately 67%. This novel inhaled LPS induced lung inflammation model in non-human primates provides a suitable model to study inflammatory processes more relevant to humans. This work also shows that inhaled CO, delivered immediately following LPS exposure, was nearly as efficacious as pretreatment with steroid at reducing neutrophil influx in the airway. Low dose inhaled CO may serve as a potential therapeutic agent in inflammatory states in humans.

**1517 EFFECT OF ETHANOL VAPORS ON PULMONARY INFLAMMATION IN A RAT MODEL OF ALLERGIC ASTHMA.**

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Ethanol is being added to fuel in various proportions in order to reduce greenhouse gas emissions. This will likely result in involuntary exposure to ethanol vapors. We have shown that ethanol vapors do not cause pulmonary toxicity in normal and ALDH2-deficient rats. The presence of a pathological pulmonary condition, such as allergic asthma, however, can lead to 270 million deaths annually which may increase susceptibility to pulmonary toxicity induced by ethanol vapors. Therefore, this study aimed to investigate the inflammatory pulmonary response of inhaled ethanol in asthmatic rats using the Brown Norway rat model sensitized and challenged (14 d later) with chicken egg ovalbumin. A dose-response was performed by increasing the ovalbumin challenge concentration (0.1 to 5 %); to monitor both upward and downward changes: 1.5 % ovalbumin (approximately 30% of the maximum inflammatory response) was selected as the level to study the effect of ethanol inhalation. Bronchoalveolar lavages (BAL) performed 6 to 72 h after the ovalbumin challenge showed that inflammatory cells peaked at 48 h: eosinophils (45%), an important leukocyte in allergic asthma, lymphocytes (38%), neutrophils (13%), and lymphocytes (4%). No significant differences were observed in the total number of white blood cells, nor in eosinophils, in rats exposed to ethanol vapors (3000 ppm, 6 h/d, 14 consecutive days) at 6, 24, 36, 48 and 72 h following the ovalbumin challenge. Thus, 3000 ppm represents a NOAEL in asthmatic rats. In terms of public health, extrapolation of these results suggests that contamination of urban air with ethanol vapors from its use in fuel would not exacerbate inflammatory response resulting from allergic asthma. Given that ethanol is present in the atmosphere with other important pollutants, we are now assessing the effect of a co-exposure with ozone (0.5 and 1 ppm) and will subsequently look at co-exposures with fine particles and byproducts of ethanol combustion, such as acetaldehyde and formaldehyde. (Supported by Asser)

**1518 DIFFERENTIAL EFFECT OF LOW AND HIGH ORGANIC CARBON PARTICULATE MATTER IN POSTNATAL AND ADULT RAT AIRWAYS.**

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Unhealthy levels of particulate air pollution affect 30% of the US population. Epidemiologic studies associate particulate matter (PM) exposures with increased respiratory disease in children. Phase I and II enzymes, both key to activation and detoxification of polycyclic aromatic hydrocarbons (PAH) within PM, differentiate postnatal in the lung. PM contains organic compounds (including PAH) at various levels depending on source. In this study, we exposed 7 day old postnatal and adult rats exposed to PM of two different content (PM) and low organic content PM (DFP). Rats were exposed for a 6 hour period to 2x10^7 particles of either type and the lungs were analyzed at 24 and 48 hrs post exposure. Protein expression was determined using immunohistochemistry and airway associated gene expression was determined by quantitative real time RT-PCR on microdissected airways. There were substantial differences in airway expression of key enzymes between postnatal and adult rats. Adults had 300 fold greater expression of CYP1A1, 2 fold greater expression of glutamine cysteine ligase (GCL), a key enzyme in glutathione synthesis, yet antioxidant heme oxygenase-1 remained unchanged within the airways. FFP exposure increased CYP1B1 in both postnatal and adult rats, but the temporal pattern differed by age. FFP caused a 10 fold increase in CYP1A1 gene expression in postnatal rats only, adults were unchanged. In contrast, DFP exposure did not change CYP1A1 airway expression in either age. Both the antioxidant Nrf2 transcription factor and GCL increased de-spitely low PAH content in the DFP. We conclude that: 1) basal levels of many key cytochrome P450s are lower in postnatal rats, 2) that the temporal pattern and expression of metabolic enzymes vary depending on the organic carbon content of the exposure and the age of the animal. Funded by: NIH ES067600 and U.S. EPA RD-83241401.
1520  EFFECT OF INHALATION EXPOSURE TO MOTORCYCLE EXHAUST ON RAT HEART.

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Epidemiological studies have demonstrated that increased levels of air pollutants are associated with cardiovascular morbidity and mortality. Motorcycle exhaust (ME) is a major source of air pollutants in urban areas where motorcycles are a popular means of transportation. ME from two-stroke engines contains higher levels of chemical toxicants and carcinogens than the exhaust from diesel engines. The present study has determined the ability of ME to cause cardiotoxicity in male rats exposed to 1:10 diluted ME 2 hours daily, Monday through Friday, for 8 weeks. ME exposure produced a 34% increase in the heart weight to body weight ratio. The results of echocardiogram measurements showed that ME exposure increased left ventricle posterior wall thickness in systole and diastole by 35% and 38%, respectively. The exposure caused respective 24% and 14% decreases of left ventricle cavity diameter in systole and diastole. ME produced a 39% increase in the calculated left ventricle mass. The results of histopathological examinations indicated that ME increased left ventricle thickness and caused cardiomyofibrosis in the apex and septa of the heart. The results of biochemical studies showed that ME exposure caused a 33% decrease of glutathione content and a 40% increase of lipid peroxidation in heart. The exposure decreased glutathione S-transferase, superoxide dismutase, and glutathione peroxidase activities by 48%, 24%, and 25% in heart cytosol, respectively. ME produced a 57% decrease of cytochrome P450-dependent 7-ethoxycoumarin O-deethylase activity in heart microsomes. These preliminary results suggest that ME has the ability to cause cardiomyocyte hypertrophy and cardiac dysfunction by mechanisms involving oxidative stress in rats.

1521  RETENTION OF PAH-RICH SOOT PARTICLES ACCOMPANIES ALTERED LUNG GENE EXPRESSION PROFILES FOLLOWING BRIEF EXPOSURE TO COMBUSTION-DERIVED NANOPARTICLES.


Rationale: Large quantities of chemically complex, inhalable soot nanoparticles are produced by incomplete combustion of petrochemicals during transportation activities, routine flaring at refineries/petrochemical facilities, refinery accidents, pipeline sabotage and terrorist attacks. Polynuclear aromatic hydrocarbons (PAHs) adsorbed to the particles’ surfaces, have been implicated in pathological responses to particle inhalation. We report here the lung responses to brief inhalation exposures to PAH-rich soot (BDS) nanoparticles produced during incomplete combustion of the high volume petrochemical, 1,3-butadiene (BD). Methods: Young adult mice and rats following the EPA series 870.7800 guideline. Exposure concentrations of 0, 500, 1000 and 2000 mg/m^3 jet fuel kerosene were targeted. The low level was designed to be vapor only, the intermediate and high levels were a mixture of aerosol and vapor. The 500 mg/m^3 atmosphere was generated by flash evaporation. The intermediate and high levels were generated by nebulization. The concentration of test material was determined by gravimetric and GC analyses for the aerosol and vapor components, respectively. A second GC method was used to separate 10 hydrocarbon components of the vapor to assess stability of the atmosphere. Samples to measure MMAD were taken with a Sierra® cascade impactor. In a 5d pilot, the total jet fuel kerosene exposure for the low level was 500 ± 17 mg/m^3 (mean ± SEM) where 3.7 mg/m^3 was aerosol (aero) and 500 mg/m^3 was vapor (v). The total concentration for the intermediate level was 1000 ± 30 mg/m^3 (130 mg/m^3 aero, 880 mg/m^3 v) with an MMAD of 3.0 ± 2.0 and the high level was 2100 ± 63 mg/m^3 (770 mg/m^3 aero, 1300 mg/m^3 v) with an MMAD of 2.6 ± 1.7. Rats were exposed to 510 mg/m^3, 1000 mg/m^3 or 2000 mg/m^3 jet fuel kerosene. The overall mean MMAD for the aerosol was 2.7 ± 2.9. Mice were exposed to 500 mg/m^3, 1000 mg/m^3 or 2000 mg/m^3. The overall mean MMAD for the aerosol was 2.9 ± 2.0. GC analysis of 10 components demonstrated that the atmospheres were stable over the exposure period. These results demonstrate that the exposure atmosphere was consistent over the course of the study, aerosols generated were respirable and the vapor phase of test material was stable.

1522  ELECTROCARDIOGRAPHIC AND AUTONOMIC EFFECTS OF ACUTE PARTICULATE MATTER (PM) EXPOSURE IN A RAT MODEL OF CARDIOMYOPATHY.


Human exposure to ambient PM from fossil-fuel emissions is linked to cardiovascular disease and death. This association strengthens in people with preexisting cardiac disease—especially heart failure (HF). Cardiomyopathy is the most common cause of HF. The mechanisms explaining PM-induced exacerbation of HF are unclear. Some of PM’s effects have been attributed to transition metal components. Residual oil fly ash (ROFA), a waste product of fossil fuel combustion from boilers, is rich in the transition metals Fe, Ni, and V, and when released as a fugitive particulate, contributes to ambient fine particulate air pollution. We hypothesized that PM exposure would exacerbate cardiopulmonary responses in a rat model of cardiomyopathy. Cardiomyopathy was induced in Spontaneously Hypertensive Heart Failure (SHHF) rats via continuous infusion with isoproterenol. Five days later, rats were exposed by nose-only inhalation for 4 h to either filtered air or 580 μg/m^3 of the PM1.0 fraction of a synthetic PM (dried salt solution consisting of Fe, Ni and V sulfates) that is similar in composition to ROFA. During PM exposure, rats with cardiomyopathy had increased bradycardia and PR interval as well as decreased heart rate variability, systolic blood pressure, and Q-wave amplitude relative to air-exposed rats with cardiomyopathy. 24-hours after PM exposure, pulmonary inflammatory inductus occurred only in rats with cardiomyopathy. Thus, acute PM exposure exacerbated cardiopulmonary injury in rats with cardiomyopathy, suggesting that this model may help elucidate the mechanisms by which PM exposure exacerbates HF. ECG data is currently being analyzed for post-inhalation effects on heart rate variability, cardiac arrhythmias, and ECG morphology. (Abstract does not reflect U.S. EPA policy; Supported by UNC/U.S. EPA CR83323601.)

1523  JET FUEL KEROSENE ATMOSPHERE GENERATION AND VALIDATION FOR MICE AND RAT IMMUNOTOXICITY STUDIES.

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Jet fuel kerosene is a derivative of crude oil refining that is a complex mixture of aromatic and aliphatic hydrocarbons. The purpose of the this study was to validate exposure atmospheres for subsequent 28-day immunotoxicity studies in female mice and rats following the EPA series 870.7800 guideline. Exposure concentrations of 0, 500, 1000 and 2000 mg/m^3 jet fuel kerosene were targeted. The low level was designed to be vapor only, the intermediate and high levels were a mixture of aerosol and vapor. The 500 mg/m^3 atmosphere was generated by flash evaporation. The intermediate and high levels were generated by nebulization. The concentration of test material was determined by gravimetric and GC analyses for the aerosol and vapor components, respectively. A second GC method was used to separate 10 hydrocarbon components of the vapor to assess stability of the atmosphere. Samples to measure MMAD were taken with a Sierra® cascade impactor. In a 5d pilot, the total jet fuel kerosene exposure for the low level was 500 ± 17 mg/m^3 (mean ± SEM) where 3.7 mg/m^3 was aerosol (aero) and 500 mg/m^3 was vapor (v). The total concentration for the intermediate level was 1000 ± 30 mg/m^3 (130 mg/m^3 aero, 880 mg/m^3 v) with an MMAD of 3.0 ± 2.0 and the high level was 2100 ± 63 mg/m^3 (770 mg/m^3 aero, 1300 mg/m^3 v) with an MMAD of 2.6 ± 1.7. Rats were exposed to 510 mg/m^3, 1000 mg/m^3 or 2000 mg/m^3 jet fuel kerosene. The overall mean MMAD for the aerosol was 2.9 ± 2.0. GC analysis of 10 components demonstrated that the atmospheres were stable over the exposure period. These results demonstrate that the exposure atmosphere was consistent over the course of the study, aerosols generated were respirable and the vapor phase of test material was stable.

1524  LUNG GLUTATHIONE ADAPTIVE RESPONSES TO CIGARETTE SMOKE DECLINE WITH AGE.

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Cigarette smoke (CS) is the leading cause of chronic obstructive pulmonary disease (COPD), accounting for more than 90% of cases. COPD, which includes emphysema, occurs in individuals after decades of smoking, making it difficult to study. In animal models of CS, mice are typically exposed to CS daily for at least 6-8 months before mild signs of emphysema start to occur, suggesting an innate adaptability that can be maintained over time. One of the main defenses against the oxidant burden caused by CS is the GSH adaptive response in the lung epithelial lining fluid (ELF) and tissue. However because mice are exposed for at least 6-8 months to develop emphysema, there may also be a secondary aging factor that contributes to the decline of the adaptive response. Therefore we tested whether there is a diminished GSH adaptive response to CS in aged animals, compared to the development of emphysema over time with chronic exposures. In the present study the GSH adaptive response was examined in both young (2 months) and aged (8, 13, 19, and 26 months) mice after an acute 5 h exposure to CS. After a single exposure to CS young mice have a robust increase in ELF GSH with levels elevated.
from 100 μM in air controls to 650 μM in CS exposed mice. In contrast beginning at 8 months of age, ELF GSH basal levels declined to 65 μM and the CS induced ELF GSH response is diminished with levels only reaching 230 μM. Furthermore, in mice 13 months of age and as old as 26 months, basal ELF GSH is reduced to 50 μM and the CS induced ELF GSH response only reaches 115 μM. In addition to the diminished ELF GSH response, there are age relate declines in tissue GSH stemming from lower expression of glutamylcysteine ligase (GCL), the key enzyme in GSH synthesis, and an inability for the aged mice to increase GCL expression in response to CS comparable to younger mice. These data suggest an age factor that has previously been overlooked but becomes a major contributing factor with chronic smoke exposures associated with lung emphysema changes.

1525 DIFFERENTIAL EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL (Δ9-THC) ON CD40 LIGAND (CD40L) EXPRESSION IN ACTIVATED HUMAN PERIPHERAL BLOOD CD4+ T CELLS.

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We have previously shown that Δ9-THC, a plant-derived cannabinoid, differentially affects the expression of CD40L on activated mouse CD4+ T cells in a stimulus-dependent manner. Even though mouse and human immune systems are similar, they differ significantly in terms of development, activation, and response to immune challenges. It has been well established that the optimum expression of CD40L on activated T cells requires binding of two transcription factors, the nuclear factor of activated T cells (NFAT) and early growth response-1 (Egr-1), to their response elements in the CD40L promoter. Previous studies from our laboratory demonstrated that the mechanism of immune suppression by Δ9-THC involves, at least in part, the impairment of NFAT activation. Thus, the objective of this study was to investigate the effect of Δ9-THC on the up-regulation of cell surface CD40L expression on activated human CD4+ T cells using different T cell stimuli in human peripheral blood mononuclear cells as well as to characterize the involvement of NFAT and Egr-1 in Δ9-THC-mediated suppression of CD40L expression. Similar to what we observed in mice, as determined by flow cytometry, pretreatment with Δ9-THC significantly suppressed the up-regulation of cell surface CD40L expression on human CD4+ T cells induced by anti-CD3/CD28, but not by phorbol ester plus calcium ionophore (PMA/IO). Time course studies demonstrated that the peak binding of NFAT or Egr-1 to their response elements derived from human CD40L promoter was 30 min, as determined by gel shift assay. Future studies will include characterization of the effect of Δ9-THC on the NFAT or Egr-1 binding activity as well as the differential effect of Δ9-THC in the presence of different T cell stimuli. (Supported in part by DA07908 and Royal Thai Government Scholarships)

1526 Δ9-TETRAHYDROCANNABINOL IMPAIRS CYTOTOXIC T LYMPHOCYTE EFFECTOR FUNCTION DUE IN PART TO ABERRANT EARLY T CELL SIGNALLING INDEPENDENT OF CANNABINOID RECEPTORS 1 AND 2.

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Δ9-Tetrahydrocannabinol (Δ9-THC) belongs to a class of over 60 structurally congeners termed cannabinoids. Although Δ9-THC is well established as immunosuppressive, the molecular basis remains elusive. In this study we used an in vitro alloimmune model of cytotoxic T lymphocyte (CTL) elicitation in which T cells differentiate into an effector phenotype in response to a hapten peptide mismatch. Splenocytes (SPLC) from C57Bl/6 (WT) mice were elicited against irradiated P815 cells. CTL activity was assessed by 51Cr release of P815 target cells. CTL activity was suppressed by Δ9-THC in a concentration-dependent manner but only when present during elicitation. Consequently, we sought out to determine the role of the cannabinoid receptors 1 (CB1) and 2 (CB2) in the Δ9-THC-mediated suppression of CTL function. Using CB1/CB2 null mice (KO), Δ9-THC suppressed CTL responses in SPLC from KO with a similar magnitude as WT. In the absence of Δ9-THC, the CTL response in KO was lower than in WT, suggesting an intrinsic role of CB1 and/or CB2 in elicitation of CTL. To explain these findings, we focused on earlier events in the allogeneic response, including expression of activation markers CD69 and CD25. In CD8+ T cells, CD69 expression was induced as early as 6 hours, while CD25 expression was induced on CD4+ T cells 24 hours after co-culture with P815. Surprisingly, Δ9-THC increased CD69 expression on CD8+ T cells and CD25 expression on CD4+ T cells. Also, CD69 and CD25 expression were induced on T cells from KO compared to WT mice. Collectively, these results indicate the inherent involvement of cannabinoid receptors in CTL elicitation as well as CB1 and CB2 independent Δ9-THC-mediated suppression of CTL function as early as 6 hours after immune stimulation. Supported by NIH DA07908.

1527 ENDogenous CANNABINOIDS REGULATE IMMUNE FUNCTIONS THROUGH INDUCTION OF MYELOID-DERIVED SUPPRESSOR CELLS.

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Cannabinoids are a group of compounds that mediate their physiological and behavioral effects by activating specific cannabinoid receptors. Cannabinoid receptor 1 (CB1) is primarily expressed in the CNS. In contrast, cannabinoid receptor 2 (CB2) is predominantly expressed on immune cells. In addition to the exogenous cannabinoids found in the Cannabis plant, there are also endogenous cannabinoids (endocannabinoids), such as 2-arachidonoyl glycerol (2-AG) and N-arachidonoyl ethanolamine (anandamide, AEA). The endocannabinoids also mediate their effects by activating CB1 and CB2 receptors. Cannabinoids have been shown to act as potent immunosuppressive agents and have been shown to mediate beneficial effects in a wide range of inflammatory diseases. In contrast, we have also shown previously that cannabinoids facilitate the growth and metastasis of tumors that fail to express CB receptors. In the current study, we tested the hypothesis that the increased tumor growth caused by cannabinoids may result from induction of CD11b+/Gr1+ Myeloid-Derived Suppressor Cells (MDSCs). Intraperitoneal injection of 2-AG or AEA led to a significant increase in MDSCs in a dose-response manner at concentrations of 5, 10, and 20 mg/kg body weight. The MDSCs can be classified into two subsets, granulocytic or monocytic based on the expression of Ly6C and Ly6G. Upon analysis of Gr-1 subtypes, these cells were shown to be both Ly6C and Ly6G double-positive thereby suggesting the induction of granulocytic version of MDSCs. MDSCs inhibit T-cell function by reducing the availability of L-arginine. Our studies revealed that the MDSCs triggered by endocannabinoids were arginase positive, a hallmark of MDSCs. Together, these studies suggest that endocannabinoids may regulate the functions of immune system through induction of MDSCs.

1528 Δ9-TETRAHYDROCANNABINOL MODULATES ANTI-HIV GP120-SPECIFIC CYTOTOXIC T LYMPHOCYTE (CTL) FUNCTION.

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Marijuana is used by HIV patients who are not undergoing antiretroviral therapy (ART) to stimulate appetite and thus attenuate the AIDS associated wasting syndrome. Δ9-Tetrahydrocannabinol (Δ9-THC) is the predominant psychoactive compound in marijuana and is known to modulate immune responses. It has been shown to both positively and negatively modulate IL-2 expression by T cells depending on the magnitude of T cell activation. Whether smoking marijuana affects the immune system for presenting antigen to T cells has been established. Using a transgenic system the gp120 gene was inserted into the genomes of a dendritic cell line, DC2.4, and a T cell line, EL-4. gp120 clones were isolated by cloning by limiting dilution, and identified by RT-PCR and intracellular staining for high gp120 expression. DC2.4gp120 clones served as antigen presenting cells (APC) to elicit T cell response, and EL-4gp120 clones served as target cells in the effector phase. Five days after co-culture of naïve splenocytes from C57Bl/6 mice with DC2.4gp120 clones or EL-4gp120 clones, CTLs were assayed for activity using IFN-γ ELISPOT enumerating gp120-specific CTLs. Higher
numbers of IFN-γ producing cells were observed when EL-4Δgp120 clones were used as target cells compared to parental EL-4 cells, and the response was modulated by Δ9-THC when introduced in the elicitation phase. Collectively, these results suggest that DC2.4gp120 clones are capable of eliciting a gp120-specific CTL response, and Δ9-THC can modulate the elicitation of CTL. Supported by NIH DA07908

1530 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) SUPPRESSES LPS-ACTIVATED BINARY SWITCHING OF B CELLS TO PLASMA CELLS.

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Impairment of B cell differentiation by TCDD is well established. Based on the gene regulatory network that underlies B cell differentiation, we hypothesized that a transcriptional bistable switch underlies B cell activation and TCDD disrupts the switching process. A computational model of the network shows that this switch can generate the two mutually exclusive transcriptional profiles corresponding to the B cell and plasma cell states. Using flow cytometry lipopolysaccharide (LPS)-activation yields two distinct cell subsets (a bimodal distribution), supporting the idea that a transcriptional bistable switch underlies B cell activation and TCDD disrupts the gene regulatory network that underlies B cell differentiation, we hypothesized that TCDD reduces DC function, and suggest that environmental factors may contribute to differential susceptibilities and responses to respiratory viral infections. (Supported by NIH Grants K02-ES012409 and R01-ES013958 to BPL, EHSC P50-ES01247, and T32-ES07026 to JLH).

1533 CHARACTERIZATION OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN EFFECT ON THE CD40L-INDUCED IGM ANTIBODY RESPONSES IN PRIMARY MOUSE AND HUMAN B LYMPHOCYTES.

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Previous studies using rodent models established the B cell as a direct cellular target of 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) in suppression of the primary antibody response, a sensitive endpoint of TCDD-mediated immune dysfunction. However, data concerning TCDD effects on human B cells remain limited, preventing a comprehensive evaluation of human risk posed by TCDD exposure on
humoral immunity. Using a recently established in vitro IgM response model, proliferation, activation, and differentiation of mouse splenic and human peripheral blood B cells into IgM-secreting cells can be induced by cell-surface expressed human CD40L plus interleukin (IL)-2, IL-6, and IL-10, mimicking a T-cell-dependent humoral immune response. TCDD suppressed the CD40L-induced IgM response in mouse B cells from C57BL/6 mice at concentrations previously reported to suppress the anti-sheep erythrocyte IgM AFC response. The mRNA levels of AHR-responsive genes cytochrome P450 1A1 (CYP1A1), CYP1B1, and TCDD-induced poly (ADP-ribose) polymerase (TPARP) were induced by TCDD in resting human B cells, confirming the functionality of the AHR signaling cascade. Preliminary studies also demonstrated that CD40L-induced IgM response in human B cells from limited number of donors was suppressed by TCDD, although the sensitivity varied among these donors. Moreover, based on donors assessed thus far, naive human B cells exhibited greater sensitivity to TCDD when compared with the total B cell population, which includes naive and memory B cells. Collectively, this series of studies provided evidence that the CD40L-induced IgM response model can be utilized to characterize the sensitivity of human B cell effector function to TCDD, and applied toward elucidating the molecular mechanism by which TCDD influences the plasticmy differentiation in mouse and human B cells. Supported in part by NIH P42 ES04911 and the Dow Chemical Company.

1534 EXPOSURE TO TCDD INCREASES EXPRESSION OF MONOCYTE CHEMOTACTIC PROTEIN (MCP)-1 AND CYCLOOXYGENASE (COX)-2 DURING LIVER REGENERATION,
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Halogenated aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are potent environmental contaminants that elicit toxicity by activating the aryl hydrocarbon receptor. Although the mechanism of toxicity remains unclear, exposure to TCDD has been shown to enhance inflammatory responses and alter cell proliferation and differentiation. Using a mouse model of liver regeneration following 70% partial hepatectomy (PH), we have previously shown that exposure to TCDD suppresses hepatocyte proliferation during liver regeneration. In the present study, we tested the hypothesis that TCDD treatment accelerates inflammation in the regenerating liver by enhancing macrophage recruitment and activation. C57BL/6 mice were treated with TCDD (20 μg/kg body weight) or vehicle (peanut oil) 24 hr prior to PH. Plasma and remnant liver tissue were collected 0-96 hr after surgery. Exposure to TCDD increased plasma levels of the monocyte chemotactant protein (MCP)-1 36 hr after surgery. However, TCDD treatment did not increase the local production of MCP-1 in the liver, nor did it increase the number of F4/80-positive macrophages in the regenerating liver. Nevertheless, heaptic expression of cyclooxygenase (COX)-2 was markedly elevated in TCDD-treated mice 24 hr after surgery. Exposure to TCDD increased plasma levels of the monocyte chemotactant protein (MCP)-1 36 hr after surgery. However, TCDD treatment did not increase the local production of MCP-1 in the liver, nor did it increase the number of F4/80-positive macrophages in the regenerating liver. Nevertheless, heaptic expression of cyclooxygenase (COX)-2 was markedly elevated in TCDD-treated mice 24 hr after surgery. Based on these results, increased expression of MCP-1 in TCDD-treated mice does not result from overproduction of this chemokine in the liver and does not correlate with enhanced recruitment of macrophages to the liver during regeneration. Moreover, it is likely that the early increase in COX-2 expression occurs independently of MCP-1 overproduction in TCDD-treated mice. Increased COX-2 expression suggests that TCDD treatment may modulate prostaglandin synthesis and prostaglandin signaling pathways, which may enhance inflammation and contribute to the suppression of liver regeneration observed in TCDD-treated mice.

1535 GENERATION OF TCDD-INDUCED REGULATORY PHENOTYPES IN BONE MARROW-DERIVED DENDRITIC CELLS.
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TCDD is the prototypical AhR ligand and a potent immunotoxicant. However, the mechanisms underlying TCDD-induced immune suppression remain to be defined. Recent evidence suggests that TCDD induces the generation of regulatory immune cells. Dendritic cells (DCs) are professional antigen presenting cells (APCs) that constitutively express the AhR and are sensitive to TCDD-induced AhR activation. DCs exposed to TCDD may have an altered APC function, causing aberrant T cell polarization. We hypothesize that TCDD-induced AhR activation in DCs increases the expression of regulatory elements giving rise to regulatory leukocytes. To test this hypothesis, “inflammatory” and “steady-state” bone marrow-derived DCs (BM-DCs) from C57BL/6 mice were grown in the presence of 10nM TCDD or vehicle. TCDD altered the immature phenotype of both inflammatory and steady-state DCs. TCDD-BMDCs displayed decreased surface expression of CD11c, while increasing CD86 expression. MHC class II expression was increased in inflammatory TCDD-BMDCs, while decreased in steady-state TCDD-BMDCs. Following LPS stimulation, inflammatory TCDD-BMDCs increased IL-6 and TNFα production, whereas steady-state TCDD-BMDCs secreted less IL-6, TNFα and IL-10. Inflammatory TCDD-BMDCs significantly up-regulated expression of the regulatory genes IDO1, IDO2 and TGFβ3, while steady-state TCDD-BMDCs up-regulated only IDO2. The expression of regulatory genes was exacerbated in both inflammatory and steady-state TCDD-BMDCs following LPS stimulation. TCDD-BMDCs from AhR−/− mice showed minimal surface marker changes and no increases in regulatory gene expression. Taken together TCDD-induced AhR activation alters DC differentiation and generates related but distinct regulatory phenotypes in inflammatory and steady-state BMDCs. Future experiments will examine if TCDD-induced regulatory DCs contribute to the observed immune suppression following TCDD exposure. This research was supported by grants from NIHES (ES015784) and NCRR (RR17670).

1536 ROLE OF SPECIFIC PROTEIN BINDING MOTIFS IN TCDD-INDUCED ACTIVATION OF THE HUMAN POLYMORPHIC HS1, 2 ENHANCER.
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The immunoglobulin heavy chain (IgH) gene is transcriptionally regulated in part by the 3′ IgH regulatory region (3′IghHR) which is located downstream of the IgH locus and in humans consists of three enhancers (hs1, hs2, hs4). Utilizing a well-characterized mouse B-cell line (CH12.LX), our previous results have demonstrated a sensitive inhibition of the mouse 3′IghHR by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), a known disrupter of B-cell differentiation, which correlated well with TCDD-induced inhibition of IgH expression and Ig secretion. Interestingly, in humans a polymorphism of the hs1,2 enhancer (resulting in a varying number of tandem repeats of a 53 bp sequence) has been correlated with several autoimmune diseases. The human hs1,2 enhancer is also sensitive to TCDD-induced modulation but in contrast to the mouse hs1,2 and 3′IghHR, TCDD activated the human hs1,2 enhancer perhaps through altered binding to one or more transcription factor binding sites within the hs1,2 enhancer (i.e., DRE, Kβ, AP-1, Oct, SP1). The purpose of the current study was to elucidate the transcriptional regulation of the human polymorphic hs1,2 enhancer following TCDD induction. Utilizing site-directed mutagenesis, mutated luciferase reporter plasmids were designed containing a variety of binding site deletions (DRE, Oct, Kβ, Oct+53bp). These plasmids were transiently transfected into the CH12.LX cells then treated with TCDD in the absence or presence of lipopolysaccharide (LPS) stimulation. Deletion of the DRE site showed a modest increase in TCDD-induced activity; whereas deletion of both the Oct site and the 53bp repeat resulted in a complete loss of TCDD-induced activation. These results suggest that there is a concerted activation of the polymorphic hs1,2 enhancer involving Oct and a site within the 53bp repeat that may be independent of the DRE. Studies are ongoing to evaluate the role of the AP-1 site in relation to these effects. (Supported by NIEHS ES015014676)

1537 EXPOSURE TO TCDD DECREASES SPLEEN CELLULARITY DURING LIVER REGENERATION.
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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent and persistent environmental contaminant that elicits toxicity by activating the aryl hydrocarbon receptor. The toxic effects associated with TCDD exposure are diverse and include immunotoxicity, enhanced inflammation, and dysregulated cell cycle control, although the mechanisms are poorly understood. We have previously shown that exposure to TCDD suppresses liver regeneration following 70% surgical partial hepatectomy (PH). Recent reports suggest that liver regeneration is negatively regulated by the innate immune system through interferon (IFN)-γ and natural killer (NK) cells, both of which are altered by TCDD treatment in other model systems. In this study, we tested the hypothesis that exposure to TCDD exacerbates IFNγ production and the expansion of NK cell numbers during liver regeneration. Mice were treated with TCDD (20 μg/kg) or vehicle 24 hr prior to PH and euthanized 12-48 hours after surgery. Lymphocytes from the spleen and remnant liver were analyzed by flow cytometry, and IFNγ was measured in liver and plasma. Contrary to our hypothesis, exposure to TCDD did not increase IFNγ protein levels in the regenerating liver, and plasma IFNγ levels were below the limit of detection. However, the number of NK cells in the regenerating liver was slightly increased in TCDD treated mice. Furthermore, TCDD treatment decreased the number of NK1.1+, CD3+, CD4+ and CD8+ lymphocytes in the spleen 12 hours after PH.
These data indicate that TCDD treatment may facilitate the evacuation or deletion of immune cells from the spleen during regeneration. Such alterations may culminate in increased recruitment of NK cells to the liver or aberrant cytokine production, either of which could attenuate liver regeneration.

**1538 ROLE OF THE ARYL HYDROCARBON RECEPTOR IN TCDD-INDUCED ALTERATION OF IMMUNOGLOBULIN EXPRESSION.**

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Dioxin exposure is known to cause chloracne, hepatotoxicity, and immune suppressive effects. The prototypic compound for studying dioxin toxicity is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits immunoglobulin (Ig) secretion in B cells. TCDD-induced inhibition of Ig secretion is thought to be modulated in part through transcriptional down regulation of the Ig heavy chain (IgH) locus. Although several regulatory elements control the IgH locus our research focuses on the 3'IgH regulatory region (3'IgHRR) that contains dioxin responsive elements (DRE) in two of its constituent hypersensitive regions, hs1, 2 and hs4. The heterodimer of the aryl hydrocarbon receptor (AhR) and the aryl hydrocarbon nuclear translocator (ARNT) binds to DREs upon ligand (i.e. TCDD) activation. In previous luciferase reporter studies TCDD treatment in LPS-activated B cells up-regulates the hs4 region but down-regulates the 3'IgHRR. Moreover, the hs4 region contains an overlapping DRE and kB motif that have been proposed to act in concert to regulate the hs4 enhancer. The purpose of the current study is to develop an AhR-deficient model in the well characterized CH12.LX mouse B-cell line in order to further elucidate TCDD-induced AhR regulation of the 3'IgHRR and its enhancers. Stable lentiviral-mediated insertion of two shRNA constructs targeting AhR message achieved approximately 50% AhR knockdown in a heterogenous population of cells as verified by Western blot analysis. Furthermore, activity of the hs4 enhancer following LPS and TCDD co-treatment was significantly reduced in the AhR-deficient cell population. These results suggest that the AhR plays a significant role in activation of the hs4 enhancer. Further, current studies are focused on utilizing this AhR-deficient model to determine the role of the AhR in TCDD-induced modulation of the 3'IgHRR which should significantly contribute to understanding the mechanism behind Ig transcription and B-cell function by TCDD. (Supported by NIEHS R01ES014676 and NIEHS Supplement for Undergraduate Research Experience)

**1539 NF-kB/REL PROTEINS ROLE IN MODULATING THE 3'IgHRR BY LPS, CpG, AND TCDD.**

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Transcriptional regulation of the immunoglobulin heavy chain (IgH) gene involves several regulatory elements including the 3'IgH regulatory region (3'IgHRR). The 3'IgHRR is composed of at least four enhancers (hs3A; hs1,2; hs3B; hs4) and contains binding sites for several transcription factors including NF-kB/Rel proteins. The dioxin responsive element (DRE) may also contribute to 3'IgHRR regulation. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a known disrupter of B-cell differentiation, induces binding of the aryl hydrocarbon receptor (AhR) to a DRE motif within both the hs1,2 and hs4 enhancers. Interestingly, in transient luciferase reporter studies TCDD profoundly inhibited 3'IgHRR activation by the B-cell activator lipopolysaccharide (LPS) but enhanced activation of the hs4 enhancer. Within the hs4 enhancer, the DRE overlaps an NF-kB/Rel binding site and site-directed mutational analysis demonstrated a cooperative interaction between proteins binding to these motifs. The objective of the current studies was to evaluate the expression of NF-kB/Rel proteins including their negative regulator IκBz following LPS stimulation and TCDD treatment and to compare these results to 3'IgHRR activity under the same treatment conditions. For these studies we utilized the CH12.LX B-cell line and its variant, CH12.1kBzA, which expresses an inducible IκBz super repressor (IκBzAA). Western blot analysis of LPS stimulated CH12.1kBzAA cells demonstrated a decrease in endogenous IκBz with a reciprocal increase in nuclear RelA which appeared to be diminished when IκBzAA was expressed. Utilizing transiently expressed luciferase reporters, induction of IκBzAA expression partially attenuated both an LPS-induced or a CpG-induced activation of the 3'IgHRR and hs4 following a TCDD and LPS or CpG co-treatment. Co-immunoprecipitation assays with the CH12.LX cells demonstrated an interaction between the AhR and NF-kB/Rel. These results suggest that NF-kB/Rel proteins, perhaps through an interaction with the AhR, are partially responsible for 3'IgHRR modulation by LPS/CpG and TCDD. (Supported by NIEHS R01ES014676)

**1540 A DRE IN THE MULTIPLE CLONING REGION OF THE pGL3 LUCIFERASE REPORTER INFLUENCES TRANSCRIPTIONAL ACTIVITY.**

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We have utilized a variety of luciferase reporter plasmids (pGL3 backbone) to characterize the transcriptional effects of the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other aryl hydrocarbon receptor (AhR) ligands on the mouse 3′ immunoglobulin heavy chain regulatory region (3′IgHRR). The 3′IgHRR is an approximately 40 kb region and is involved in the transcriptional regulation of the immunoglobulin heavy chain (IgH) locus, the gene encoding the heavy chain protein of Ig or antibodies (i.e. secreted Ig). Utilizing luciferase reporters transcriptionally regulated by a variable heavy chain promoter and the mouse 3′IgHRR, we have previously demonstrated a profound and sensitive inhibition by TCDD of 3′IgHRR activation following lipopolysaccharide (LPS) stimulation which closely correlates with the inhibitory effect of TCDD on LPS-induced IgH gene and protein expression. More recently we have evaluated the human hs1,2 enhancer which has been associated with several human diseases. However sequencing of the human hs1,2 luciferase reporters identified a DRE core motif within the multiple cloning region (mcsDRE) of the pGL3 luciferase plasmid backbone. Therefore the objective of this study was to determine if the mcsDRE influenced reporter activity of the human hs1,2 plasmids in the well-characterized B-cell line model (CH12.LX). Deletional analysis demonstrated that TCDD-induced reporter activity was greater with the presence of the mcsDRE though the trends remained the same. These results are significant in that many chemicals have been shown to interact with the AhR and influence transcription through the DRE and presence of the mcsDRE in the pGL3 luciferase plasmid may inappropriately influence promoter and enhancer analysis. Therefore pGL3 reporters should be carefully constructed as to avoid retaining the mcsDRE core motif (GCCTGC). (Supported by NIEHS R01ES014676)

**1541 PHTHALATE (2-ETHYLHEXYL) ESTER AFFECTS IL-4 EXPRESSION THROUGH CA/NFAT SIGNALING IN SPLEEN LYMPHOCYTES.**

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Phthalate (2-ethylhexyl) ester (DEHP)’s immune toxicology is paid more attention as reports on DEHP close relation with allergic diseases began to accumulate. Studies have indicated that DEHP modulates the production of interleukin-4 (IL-4) in lymphocytes, a pro-inflammatory cytokine closely associated with allergic immune response, although the mechanism remains unclear. The scope of the present study was to analyze whether DEHP affects IL-4 production through CαNFAT signaling and whether mono-(2-ethylhexyl) phthalate (MEHP), one of DEHP’s major metabolites, contributes to that change in vitro. Primary spleen lymphocytes from BALB/c mice were exposed to DEHP(10,50 mol/L) or MEHP(40,80 mol/L) activated with 10μg/mL PMA and 0.5mg/mL Ionomycin or 25μg/mL PMA and 1mg/mL Ionomycin. mRNA expression were determined by realtime-PCR test. ELISA method or Western Blotting were used to detect protein production. IL-4 mRNA for 4h and protein for 96h were significantly increased by DEHP, which was different from the results that MEHP did not obviously affect IL-4 expression under different stimulation condition. That tazocin simultaneously suppressed DEHP-induced IL-4 enhancement indicated NFAT was involved in DEHP modulating IL-4 expression. Concordantly, no matter NFATp or NFATc mRNA expression for 4h were obviously enhanced as well as their protein by Western Blotting. Transient transfection of IL-4 cells with a luciferase gene under the control of the distal NFAT binding site from the IL-4 promoter showed increased transcriptional activity by DEHP. In addition, DEHP-mediated enhancement of calcineuron protein,NFAT and IL-4 expression were abrogated by calcium antagonist verapamil. Collectively, these results suggest that DEHP affecting IL-4 expression is mediated through Cα/NFAT signaling pathway, in which MEHP does not play a part.
Effects of brominated flame retardants (BFRs), decabrominated diphenyl ether (DBDE), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA), on host immunity of mice were evaluated using respiratory syncytial virus (RSV) infection. Five-week-old female mice were exposed to BFRs mixed with the diet at 1000 or 10,000 ppm for 28 days, and subsequently infected with RSV. No toxicological sign was observed in BFRs-exposed mice. However the pulmonary viral titers in TBBPA-exposed mice significantly increased compared with the control on day 5 post-infection, but those in DBDE and HBCD-exposed mice did not. Exacerbation of the pneumonia due to RSV infection was histopathologically observed in TBBPA-exposed mice. To evaluate effects of TBBPA on host immune response to RSV infection, the levels of various cytokines in bronchoalveolar lavage fluid (BALF) from RSV-infected mice exposed or unexposed to TBBPA were compared by ELISA. TBBPA exposure significantly increased the levels of TNF-α, IL-6 and IFN-γ in BALF 5.8, 2.6 and 5.2-fold compared with those of the control at the each optimal time point after virus infection, respectively. However, those of IL-4 and IL-10, Th-2 cytokines, significantly decreased to 0.7 and 0.8-fold of those of control, respectively. Thus, TBBPA exposure caused the unusual production of various cytokines in RSV-infected mice. A flow cytometric analysis revealed that percentage of CD4-positive/CD8-positive cells, immature T lymphocytes, to the cell populations in BALF from RSV-infected mice increased 2-fold due to TBBPA exposure. The change was not observed in spleen cells of TBBPA-exposed mice. These results verified that TBBPA exposure affected the host immune response to RSV infection, such as irregular changes of cytokine production and immune cell population, suggesting that TBBPA, not DBDE and HBCD, is an immunotoxic agent.

MOLECULAR MECHANISM OF THE INHIBITION OF INNATE IMMUNE RESPONSE TO LPS BY SODIUM METHYLTHIOCARBAMATE.

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Sodium methylthiocarbamate (SMC) is the third most abundantly used conventional pesticide in the USA. One of major human health risks imposed by SMC is the inhibition of inflammation and decreased resistance to infection. Here we utilized a mouse model to characterize the molecular mechanisms of inhibition of the response to LPS via proteomic microarray. Changes were isolated from the peritoneal cavity of mice treated with SAD at 100, 200, and 300 mg/kg, 15 min before administration of LPS (60 μg/mouse). Cells were harvested 2 hr after LPS treatment. LPS-induced gene expression changes significantly altered by SAD treatment were further examined for their biological functions. Among these genes, toll like receptor 4 (TLR4) and CD180 were overexpressed in the SAD group. Since NF-κB has been implicated in significant cellular functions involved in immune system and stress responses, these results suggest that the function of B cells may be impaired by SAD, leading to inhibition of the innate immune response to LPS.

MODULATION OF NF-κB PATHWAY BY GOLD NANOPARTICLES IN B CELLS.

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The transcription factor NF-κB has been shown to orchestrate a wide spectrum of cell, tissue and organ level responses. Almost all cells express NF-κB, which can be activated or inhibited in response to over 200 chemical, physiological and environental stimuli. Gold compounds (for instance, aurothiomalate and aurothioglucone) have been shown to block the activity of IkB kinase, through their interaction with a cysteine residue on the enzyme, leading to inhibition of NF-κB activation. Since gold nanoparticles (colloidal gold) also have an affinity for thiol groups, this study hypothesizes that the metal sensitive IkB kinase can act as a potential target for gold nanoparticles to bind and modulate the activity of NF-κB. This study aims at providing a better understanding of how gold nanoparticles affect the NF-κB signaling pathway and modulating the pathway using silicon coated gold nanoparticles functionalized with DNA, using a murine B cell line as the model system. Silicon coated gold nanoparticles still retain the unique optical properties of the gold, but lose their inherent chemical reactivity towards thiol groups. These bio-compatible nanoparticles can then be functionalized with DNA to specifically alter the NF-κB pathway and modulate cellular function. Preliminary data, using reporter assays, suggested that gold nanoparticles downregulate the activity of NF-κB in B cells and increased the mitochondrial function in the B cell line being tested. Since NF-κB has been implicated in significant cellular functions involved in immune system and stress responses, these results suggest that the function of B cells and perhaps other cells systems could potentially be compromised by gold nanoparticles. With the ever expanding list of biomedical applications such as biosensors, real time monitoring of cellular environment, it is especially important to assess the impact of gold nanoparticles on the cellular functions before they can be safely used for nanomedicine.

ENDOSULFAN-α ENHANCES LPS-STIMULATED CYTOKINE PRODUCTION IN RAW 264.7 CELLS.

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Endosulfan is an organochlorine insecticide of the cyclodiene group that is used in numerous countries including the United States. It is typically manufactured as a mixture of endosulfan-t and endosulfan-β. Endosulfan has been shown to stimulate the production of inflammatory cytokines [e.g., tumor necrosis factor alpha (TNF) and interleukin-6 (IL-6)] as well as nitric oxide (NO) by the mouse macrophage cell line RAW 264.7. Because endosulfan-α, rather than endosulfan-β, has been found to be the primary mediator of some toxicities, we examined the ability of endosulfan-α to stimulate inflammatory cytokine and NO production by RAW 264.7 cells after exposure for 24 hours in culture. Using an MTT reduction assay, endosulfan-α was found to be cytotoxic (>70%) at 100 μM, but no statistically significant cytotoxicity was observed at 53 μM or lower concentrations. In the absence of E. coli lipopolysaccharide (LPS), no TNF-α, IL-6 or NO was detected using ELISA (TNF and IL-6) and Griess (NO) assays at any concentration of endosulfan-α up to 53 μM. In the presence of LPS (0.1 μg/ml) for the 24 hour culture period, TNF, IL-6 and NO levels were found to be approximately 3 ng/ml, 2 ng/ml and 30 μM, respectively. LPS-stimulated TNF levels increased more than 3-fold in the presence of endosulfan-α in a concentration-dependent manner to >10 ng TNF/ml at 33 μM endosulfan-α. Similarly, IL-6 levels increased to >5 ng/ml with endosulfan-α exposure at 33 μM. In contrast, no increase of NO levels was observed in the presence of endosulfan-α. These data indicate that exposure of RAW 264.7 cells to endosulfan-α alone does not stimulate production of TNF, IL-6 or NO, but LPS-stimulated production of TNF and IL-6 is enhanced. These data suggest that endosulfan-α may enhance some inflammatory responses.

SUPPRESSIVE EFFECT OF ASBESTOS-EXPOSURE ON THE DIFFERENTIATION INTO HUMAN CYTOTOXIC T LYMPHOCYTES.

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[Background and Purpose] Exposure to asbestos can cause malignant mesothelioma and lung cancer. In the anti-tumor specific cytotoxic T lymphocytes (CTL) differentiated from naive CD8+ T cells play a critical role. However, until now, effect of asbestos fibers on CTL has not been examined. Therefore, in this study, we investigated the effect of asbestos-exposure on differentiation of CTL. [Materials and Methods] CTL were induce by allogeneic mixed lymphocytes reactions (MLR). Human peripheral blood mononuclear cells (PBMCs) were cultured with irradiated allogeneic PBMCs with or without chrysotile B (CB) or crocidolite (CR) asbestos at 5μg/ml. After 7 days of MLR, PBMCs were assayed for the percentage and cell-number of CD8+ T cells, the expression levels of intracellular gransmze B (GB) and IFN-γ and cell-surface CD45RA, CD45RO and CD25 in CD8+ cells, apoptosis, cell-proliferation, and allogeneic cytotoxicity. On the other hand, supernatants were examined for productions of IL-10, IFN-γ, TNF-α, and IL-2 by Cytometric Beads Array. All of these were performed by flow cytometry. [Results and Discussion] Exposure to CB during MLR suppressed the increases in percentage and cell-number of CD8+ T cells in
response to allogenic cells, where PBMCs exposed to CB, but not CR, showed a
marked decrease in cytotoxicity. They also showed decreases in GB- cells, IFN-γ cells, CD45RA+ effector/memory cells and CD25+ activated cells in CD8+ cells
and an increase in CD45RA+ cells, compared with PBMCs after MLR without CB.
These results indicate that exposure to CB has a potential to suppress the differen-
tiation of CTL from naïve CD8+ T cells. CB-exposure suppressed the proliferation
in CD8+ cells without annexin V+ apoptotic cells in CD8+ cells and CD4+ cells.
The productions of IL-10, IFN-γ, and TNF-α, but not IL-2 were also suppressed by
the exposure to CB. These results deny the possible contribution of IL-10 or toxicity
of CB to the low induction of CTL. Our present study suggests that immune-sup-
pressive effect of asbestos might promote tumor disease.

1547 ASBESTOS-EXPOSURE CAUSES SUPPRESSED EXPRESSION OF NKp46 WITH LOW CYTOTOXICITY IN NK CELLS, RELATED WITH MALIGNANT MESOTHELIOMA.
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Asbestos is well known to have a tumorigenic activity, which is thought to cause
malignant mesothelioma (MM). The development of tumor is usually suppressed by
anti-tumor immunity. In particular, natural killer (NK) cells play an exsistant role in
innate immunity for tumor. However, effect of asbestos on NK cells remains
to be clarified. Cytotoxicity of NK cells is regulated by activation through cell-surface
NK cell-activating receptors including NKGD2, 2B4 and NKp46. Therefore, in the
present study, we examined the effect of exposure to asbestos on cytotoxicity and expression
levels of NK cell-activating receptors in NK cells, and compared per-
ipheral blood (PB)-NK cells among healthy volunteers (HV), pleural plaque (PL)-
positive people and patients with MM. Exposure to chrysotile B (CB) asbestos during
the culture of PBMCs suppressed the cell-surface expression of NKp46, but not
NKGD2 or 2B4, on NK cells, whereas crocidolite asbestos or glass wool did not.
NK cells purified from CB-exposed PBMCs showed the decreased mRNA
of NKp46 and the not increased level of intracellular NKp46. They showed the low
cytotoxicity for K562 cells and NKp46-mediated targets. The decreased expression
of NKp46 was also observed in NK cells interacting with the CB-exposed culture of PBMCs via membrane. Cytotoxicity of PB-NK cells was correlated with level of
NKp46, but not NKGD2 or 2B4. PB-NK cells of MM-patients showed lower cy-
toxicitv and NKp46 level compared with HV. Although cytotoxicity and NKp46
level did not differ between PL- and HV-groups, NKp46-low PL-group showed
lower cytotoxicity than NKp46-high PL-group. Finally, cytotoxicity of NK cells
was inversely correlated with the scores of 1, 2 and 3 assigned to NKp46-high PL-
, NKp46-low PL- and MM-groups, respectively. These results indicate that exposure
to chrysoite asbestos causes decreased cytotoxicity with suppressed expression of
NKp46 gene, and suggest that NKp46 might be a possible marker for impaired
anti-tumor immunity in people exposed to asbestos.

1548 FUNCTIONAL ANALYSIS OF HUMAN CD4+ T CELLS WITH INCREASED CXCR3 EXPRESSION BY LONG-TERM, LOW-LEVEL EXPOSURE TO ASBESTOS.
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[Introduction] Chronic exposure to asbestos results in pleural plaque (PP) and ma-
lignant mesothelioma (MM). To identify molecular markers predicting the im-
mune status of these patients, we examined the gene expression of human adult T
lymphocyte in the liver. In conclusion, APAP-induced liver injury is attenuated by poly(I:C) and increased
expression in CD4+ T cells, and might lead to the decline of anti-tumor immunity
in patients with PP and MM.

1549 TRIBUTYL Tin: B CELL TOXICANT AND BONE MARROW MICROENVIRONMENT MODULATOR.
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A sophisticated balance of stromal elements and B cell precursors in the bone marrow
perpetuates B cell development throughout life. Studies indicate that environ-
mentally ubiquitous peroxisome proliferator activated receptor γ (PPARγ) agonists
adversely affect developing B cells directly. However, research has just begun to ad-
dress the potential for PPARγ agonists to skew stromal cell differentiation and alter
the bone marrow microenvironment that supports B lymphopoiesis. Tributylin (TBT)
recently has been identified as a highly potent, dual PPARγ/RXR agonist and
an adipogenic agent (~10nM EC_{50}). Detection of organotins in human popu-
lations in general, suggests that the growing use of organotins in the manufacture of
plastics, wood preservatives, and pesticides has resulted in significant human ex-
posure. We hypothesize that TBT suppresses B lymphopoiesis by two mechanisms:
by inducing apoptosis in B cells directly and by altering the bone marrow microen-
vironment that supports lymphopoiesis. TBT, at nanomolar concentrations rele-
vant to human exposure, activates a mitochondrial-dependent apoptotic pathway
in a developing B cell model (a mouse pro/pre-B cell line) that is distinct from the
calcium-dependent pathway activated by exposure to micromolar concentrations.
In primary mouse bone marrow mesenchymal stem cell cultures and a bone mar-
worm stromal cell line (BMS25), TBT and the PPARγ agonist rosiglitazone, stimu-
late adipoicte differentiation, while suppressing osteoblast differentiation. Inter-
estingly, TBT has a more substantial effect on osteoblast differentiation than
adipocyte differentiation and the RXRγ agonist bexarotene suppresses osteogene-
sis, but had little effect on adipogenesis. Given that osteoblasts recently have been
shown to support B lymphocyte development and that adipocytes are suppressive,
the combination of direct stimulation of B cell death by TBT along with its ability
to skew the bone marrow components may result in significant suppression of B
lymphopoiesis.

1550 MICROBIAL COMPOUNDS ALTER ACETAMINOPHEN-
INDUCED IMMUNE RESPONSES IN THE LIVER.
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Many drugs are known to induce adverse immune reactions in susceptible individ-
uals and may result in clinical diseases. Etiology of these adverse immune reactions
is not completely understood, but known risk factors are e.g. metabolism and viral
infections. Hepatic metabolism is a key process in generating adducts forming
metabolites of orally taken drugs. Furthermore, the liver is an immunologically ac-
tive organ with a distinct lymphocyte population responsible for tolerance induc-
tion to orally administered antigens. Viral or bacterial infections in the liver could
disrupt the homeostasis by inducing liver injury and changes in lymphocyte popu-
lation, leading to the induction of immunological responses to harmless added
antigens. We have investigated the effect of LPS and poly(I-C) as model compounds
for GRAM-negative bacteria and viruses, respectively, on acetaminophen (APAP)-
induced liver injury and changes in hepatic lymphocyte population. C57Bl/6 mice
received a single oral dose of 300 mg/kg APAP and 24 hours later liver enzymes
were assessed, liver lymphocytes were isolated and analyzed, and liver histology
was evaluated. The administration of APAP elevated the plasma levels of ALT and AST.
The nonhepatotoxic regioisomer AMAP did not induce liver injury at this dose.
Furthermore, APAP treated mice had increased numbers of liver lymphocytes.
Especially the amount of neutrophils was elevated. When mice received a single
dose of LPS intraperitoneally 4 hours after APAP, APAP-induced liver injury was
increased. Strikingly, poly(I-C) injection 6 hours after APAP re-
duced both APAP-induced liver injury and changes in liver lymphocyte population.
In conclusion, APAP-induced liver injury is attenuated by poly(I-C) and increased
by LPS. These findings may have implications for systemic responses to APAP.

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The bone marrow is a multifunctional organ that supports bone formation and lymphopoiesis. Both functions are compromised during aging, and the loss of function correlates with increased fat formation within the bone marrow. A number of environmental contaminants activate the master regulator of adipocyte differentiation, the peroxisome proliferator-activated receptor γ (PPARγ), including phthalates and organotins. The pervasive use of plastics has led to increasing and detectable human exposure to both of these classes of contaminants. Tributyltin (TBT) and triphenyltin (TPhT) potently stimulate adipocyte differentiation with potentially deleterious effects to environmental PPARγ-activated adipocyte differentiation, but to a lesser extent than it inhibited rosiglitazone in cultured mouse bone marrow stromal (BMS2) cells and the role of PPARγ in their effects. Tributylin (TBT) and triphenyltin (TPhT) potently stimulated adipocyte differentiation in BMS2 cells, as visualized by Oil Red O staining and quantified by Nile Red fluorescence. The EC50 were similar to rosiglitazone, a classic PPARγ agonist (TBT and TPhT: 8X10-9M, rosiglitazone: 2X10-9M). The organotins, dibutyltin, monobutyltin and tributyltin, and the RXRα agonists, 9,10-phenanthrene and bexarotene, minimally induced adipocyte differentiation. Treatment with the PPARγ antagonist T0070907 significantly reduced TBT-induced adipocyte differentiation, but to a lesser extent than it inhibited rosiglitazone-induced differentiation. Adipocyte differentiation in BMS2 cells was associated with a significant increase in PPARγ expression, but with little change in RXRα expression, but with little change in PPARγ and RXRα expression. The biological interpretation of the microarray data was performed with Gene Set Enrichment Analysis (GSEA) using common gene sets derived from Gene Ontology, KEGG and Biocarta. GSEA of the microarray data showed that DON induced genes involved in endoplasmic reticulum (ER) stress and unfolded protein response, while all other PFCs either did not significantly change expression or affected a smaller number of genes. The statistical significance of the enrichment analysis was assessed using commercially available ELISA kits in cell-free supernatants. The data suggest that DON induces ER stress and p53-mediated apoptosis in human Jurkat cells.

Deoxyxynivalenol (DON) is a trichothecene mycotoxin also referred to as vomitoxin. Human consumption of DON-contaminated foods, particularly cereals, poses a potential health hazard and may result in adverse effects on the immune system. Although DON is known to cause ribotoxic stress in the presence of DON and its complexed with microsomal RNA, the precise mechanism underlying the immunotoxic effects of DON is not yet fully understood. To get more insight into the mode of action of DON, the human T lymphocyte cell line Jurkat was treated with two different DON concentrations (0.25 and 0.5 μM) for four time intervals (3h, 6h, 12h and 24h). Total RNA was isolated and subjected to DNA microarray analysis (Agilent 4x44k). The expression of genes involved in ribotoxic stress was determined using a panel of 20 genes. The biological interpretation of the microarray data was performed with Gene Set Enrichment Analysis (GSEA) using common gene sets derived from Gene Ontology, KEGG and Biocarta. GSEA of the microarray data showed that DON induced genes involved in endoplasmic reticulum (ER) stress and unfolded protein response (UPR) within 3h of exposure. T cell activation, proliferation, apoptosis and general stress pathways were significantly up-regulated after 3h. After 6h treatment, apoptosis and T cell activation was down-regulated. On the basis of this analysis we hypothesize that DON induces ER stress and p53-mediated apoptosis in human Jurkat cells.

PFOS and potential mechanisms attributing to suppression of humoral immunity. It is known that PFOS, along with other compounds of this class, cause peroxisome proliferation. Since lymphocytes express peroxisome proliferator activated receptor-alpha (PPAR-alpha) it has been suggested that the immunotoxicity of PFOS may be related to PPAR-alpha activation. Previous studies from this laboratory have demonstrated that PFOS suppresses IgM humoral immune responses in adult mice. To assess potential pathways of PFOS-induced immunosuppression, this study compared the profile of PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28-days with respect to the wild type control (C57Bl/6 mice; WT). Mice were exposed to PFOS for 28 days. NF-KB and PPAR-alpha binding using transcription factor ELISAs in PPAR-alpha mutant mice (MUT) with respect to the wild type control (C57Bl/6 mice; WT). Mice were exposed to PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28 days. NF-KB and PPAR-alpha binding were not altered compared to control in either species. However, after 28 days, treatment with PFOS caused a decrease in PPAR-alpha nuclear binding using transcription factor ELISAs in PPAR-alpha mutant mice (MUT) with respect to the wild type control (C57Bl/6 mice; WT). Mice were exposed to PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28 days. NF-KB and PPAR-alpha binding were not altered compared to control in either species. However, after 28 days, treatment with PFOS caused a decrease in PPAR-alpha nuclear binding using transcription factor ELISAs in PPAR-alpha mutant mice (MUT) with respect to the wild type control (C57Bl/6 mice; WT). Mice were exposed to PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28 days. NF-KB and PPAR-alpha binding were not altered compared to control in either species. However, after 28 days, treatment with PFOS caused a decrease in PPAR-alpha nuclear binding using transcription factor ELISAs in PPAR-alpha mutant mice (MUT) with respect to the wild type control (C57Bl/6 mice; WT). Mice were exposed to PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28 days. NF-KB and PPAR-alpha binding were not altered compared to control in either species. However, after 28 days, treatment with PFOS caused a decrease in PPAR-alpha nuclear binding using transcription factor ELISAs in PPAR-alpha mutant mice (MUT) with respect to the wild type control (C57Bl/6 mice; WT). Mice were exposed to PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28 days. NF-KB and PPAR-alpha binding were not altered compared to control in either species. However, after 28 days, treatment with PFOS caused a decrease in PPAR-alpha nuclear binding using transcription factor ELISAs in PPAR-alpha mutant mice (MUT) with respect to the wild type control (C57Bl/6 mice; WT). Mice were exposed to PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28 days. NF-KB and PPAR-alpha binding were not altered compared to control in either species. However, after 28 days, treatment with PFOS caused a decrease in PPAR-alpha nuclear binding using transcription factor ELISAs in PPAR-alpha mutant mice (MUT) with respect to the wild type control (C57Bl/6 mice; WT).
late suppressed LPS-induced TNF-α production both in primary human cultures as well in THP-1 cell line, while IL-8 was suppressed in THP-1, unaffected in female leukocytes and increased in male leukocytes. In THP-1 cells, we could demonstrate that the effect was pre-transcriptional, as assessed by a reduction in both LPS-induced TNF-α and IL-8 mRNA expression. A different effect of PFOA and PFOS on LPS-induced NF-kB activation could be demonstrated, suggesting a different mode of action for these PFCs. Overall, the studies support that PFCs directly suppress cytokine secretion in THP-1 cells. Among the different PFCs, PFOA appears to be the less effective compound. Acknowledgements: This research was supported in part by the Intramural Research Program of the National Institute of Environmental Health Sciences, National Institutes of Health.

1556 TH2 SKEWING BY NRF2 ACTIVATION IN CD4+ T CELLS AS EVIDENCED BY INCREASED PRODUCTION OF IL-4, IL-5, AND IL-13 AND DECREASED PRODUCTION OF INFγ

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Nuclear factor erythroid 2 related factor 2 (Nrf2) is a transcription factor that is activated by cellular stress, including oxidative and electrophilic stresses. Numerous compounds are used experimentally as Nrf2 activators, including tBHQ and BHA. Interestingly, tBHQ and BHA are also used commercially as food preservatives. Nrf2 has been reported to have anti-inflammatory activity in various models of inflammation, including septic shock and others. Whereas Nrf2 has been characterized in macrophages, much less is known about the role of Nrf2 in T cells. Accordingly, the purpose of the present studies was to ascertain the function of Nrf2 in T cells. Our studies demonstrate that tBHQ and BHA suppress INFγ production by CD3/CD28-stimulated splenocytes. The decrease in INFγ transcription by Nrf2 correlates with decreased binding to the AP-1 and NHR consensus binding sites as well as decreased nuclear accumulation of c-fos, JunD and p65 (NFκB). To determine whether inhibition of INFγ production by splenocytes activated with a T cell-specific activator is a cell-autonomous effect within T cells or whether other cell types are involved, the effect of Nrf2 activators on cytokine production by isolated CD4+ cells was assessed. tBHQ suppressed production of INFγ, a TH1 cytokine, in CD4+ cells derived from wild-type, but not Nrf2-null mice. Conversely, tBHQ stimulated production of the TH2 cytokines, IL-4, IL-5, and IL-13 in a Nrf2-dependent manner. In contrast to the TH1/TH2 cytokines, Nrf2 had little effect on production of IL-10 and TNFα. Collectively, the data suggest that tBHQ induces skewing toward the TH2 subtype through activation of Nrf2. Further studies will be needed to determine whether the addition of tBHQ to foods as a preservative may promote food allergy and/or compromise cell-mediated immunity. (Supported by NIH grants DK0811461, ES07079, and RO2019460).

1557 5-NITROAPOCYNN REVERSES LPS-, BUT NOT PMA-INDUCED IL-6 RELEASE BY MOUSE J774 MACROPHAGES: A POSSIBLE ROLE OF PROTEIN KINASE-C

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Recently we have shown that apocynin (Apo) can undergo metabolic transformation to 5-nitroapocynin (5-NitroApo) by perynitrine/CO2. In the present study we examined the effects of 5-NitroApo and Apo on the release of inflammatory cytokines, IL-6 and TNFα by mouse J774 macrophages. Pre-incubation of macrophages with 50 μM 5-NitroApo for 1 h reduced the lipopolysaccharide (LPS; 10 μM)-induced IL-6 release in the culture supernatants. Exposure to Apo also decreased the IL-6 secretion but the magnitude of this decrease was smaller than that observed with 5-NitroApo. In these experiments, 5-NitroApo was not cytotoxic to macrophages in the concentration range of 0 to 100 μM for 24 h. Phorbol myristate acetate (PMA), on the other hand, was somewhat cytotoxic to macrophages and this could not be reversed by either Apo or 5-NitroApo. In assays using PMA, 5-NitroApo but not Apo increased the release of IL-6. In both LPS and PMA exposures, macrophages showed little or no increase in the release of TNFα. Apo and 5-NitroApo, which did not alter the PMA- or LPS-induced release of TNFα, per se lowered the secretion of TNFα by macrophages. These observations suggested that Apo and 5-NitroApo inhibit the LPS-induced NFκB activation (presumably mediated via Toll-like receptor 4, TLR4) and thereby reduces the associated inflammatory response measured in terms of IL-6 release. On the other hand, the inflammatory response by PMA, mediated through activation of protein kinase-C (PKC), could be increased by 5-NitroApo. [Funding support from NIH (P20 RR16456) and Department of Education (P031B040030) is acknowledged.]

1558 TUNGSTEN INDUCES DNA DAMAGE AND ALTERS GROWTH OF DEVELOPING B LYMPHOCYTES.

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Tungsten is used widely in modern life in everything from household goods to technologically advanced material. However, little is known about the consequences of tungsten exposure. The lack of information concerning the toxicities associated with tungsten has been highlighted recently when high tungsten levels were discovered at the sites of several pediatric leukemia clusters. In these clusters, the majority of the children developed acute lymphoblastoid leukemia of the preB lymphocyte subtype. Thus, we investigated whether tungsten exposure alters the growth of preB cells and defined signaling pathways induced by tungsten. We exposed a co-culture system of the BU-11 preB cell line grown on BMS2 stromal cells to tungsten at doses found at the sites of the leukemia clusters. Growth, cell cycle, and apoptosis were determined in both cell types. While no significant changes in any of these parameters were observed in the BMS2 stromal cells, tungsten treatment for 48 hours decreased the BU-11 cell number, which correlated with an accumulation of cells in G0/G1 and increased apoptosis. Unlike polyolic aromatic hydrocarbons, tungsten induced apoptosis in BU-11 cells grown in IL-7 and therefore, is independent of the stromal cell layer. These results were confirmed in primary murine bone marrow cultures and in human peripheral blood mononuclear cells. In order to begin analysis of signal pathways induced by tungsten, cDNA microarrays were performed in a leukemic cell line following 24 hours of tungsten treatment and integrated pathway analyses were performed. Genes involved in DNA damage and ER stress were highly upregulated following tungsten exposure and validated by qPCR. An increased level of DNA damage was confirmed by COMET assay. These results suggest that the developing B lymphocyte population is sensitive to tungsten-induced toxicities and that tungsten exposure may contribute to leukemogenesis.

1559 TRANSCRIPTIONAL REGULATION OF HISTOCOMPATIBILITY COMPLEX CLASS II (MHC-II) GENES BY AGONIST AND ANTAGONIST OF PREGNANE X RECEPTOR.

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Pregnane X Receptor (PXR) is a sensor to a broad range of natural and synthetic xenobiotics to mediate the induction of CYP3A and other drug metabolizing enzymes. Accumulating evidence suggests the role of PXR in other transcriptional regulations (e.g., energy and glucose metabolism, lipid metabolism and inflammation). Adding to these findings, activation of rodent PXR induces the Major Histocompatibility Complex class II (MHC-II) in primary hepatocytes. The aim of this study is to investigate further the role of PXR and its possible involvement in the immune response. First, we determined the promoter activity of MHC-II (HLA-DRt) experiments with or without over-expression of human PXR in co-transfected HT-1080 fibrosarcoma cells. Using human bronchial epithelial cells (BEAS-2B) we measure mRNA levels of MHC-II after treatments with PXR agonist at different time intervals. To determine a possible role of PXR in the IFNγ pathway, we also used a PXR antagonist (sulforaphane) to evaluate changes in the IFNγ-mediated transcriptional regulation of HLA-DRt. HLA-DRt promoter activity was induced by activation of endogenous PXR and by activation of the IFNγ pathway in co-transfected HT-1080. Over-expression of PXR in co-transfected HT-1080 cells also induced the HLA-DRt promoter activity. In BEAS-2B cells, both HLA-DRt and PXR mRNAs were elevated after treatment with rifampicin or IFNγ. Other PXR agonists, SR12813 and dexamethasone, also up-regulated the HLA-DRt mRNA levels. The PXR antagonist, sulforaphane, inhibited the IFNγ-mediated transcriptional up-regulation of HLA-DRt after 4h and 24h in BEAS-2B. These findings support the hypothesis of a potential involvement of PXR enhancing transcriptional induction of MHC-II genes. These results continue to add to possible associations between immune and detoxification regulations through PXR as means of a xenoprotective mechanism.
Fine particulate air pollutants, mainly its organic fraction, have been demonstrated to be associated with cardiovascular and respiratory health problems. Puerto Rico has been reported to have the highest prevalence of pulmonary diseases (e.g., asthma) in the US. Organic extracts from air-borne particulate matter (PM2.5) collected in Puerto Rico throughout an 8-month period were pooled (composite) in order to perform chemical analysis and biological activity testing. Our previous findings showed that these PM2.5 organic extracts contain toxic as well as bioactive components that regulate the secretion of cytokines and the transcription of the MHC-II in human bronchial epithelial cells (BEAS-2B). The aim of this study was to assess the metal content in these extracts using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Trace elements analyses confirmed the presence of metals in the organic extracts, highlighting the relative high abundance of Cu and Zn in polar organic extracts that correlate with the induction of MHC-II transcription and secretion of IL-6 and IL-1β proinflammatory cytokines by the same extracts. BEAS-2B cells were exposed to copper sulfate pentahydrate (CuSO4) to assess cytokotoxicity and relative gene expression of MHC-II, IκBα, and CYP3A5. CuSO4, at concentration as high as 100μg/mL, did not cause cytokotoxicity, but it seems to regulate the MHC-II transcription in BEAS-2B. This research states the grounds for future studies to explain the effects of specific components in organic extracts of PM2.5 from different areas of Puerto Rico and to elucidate possible signaling pathways in the development of respiratory inflammatory diseases.

**1560** ASSOCIATION OF COPPER TO IMMUNOLOGICAL MARKERS RESULTING FROM EXPOSURE TO POLAR ORGANIC EXTRACTS FROM AIRBORNE FINE PARTICULATE MATTER (PM2.5) FROM PUERTO RICO IN BEAS-2B CELLS.

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Transdermal delivery may expose the epidermis and underlying structures to drug concentrations greater than previously tested in nonclinical studies by alternate routes. To assess the skin exposure for safety evaluation, ADE studies were performed comparing the donepezil transdermal patch with an oral donepezil dose previously tested in the 2-year rat carcinogenicity study, in which no test article-induced tumors were found. Quantitative Whole Body Autoradiography (QWBA) comparison in Long-Evans rats administered 14C-donepezil either as single oral dose (30 mg/kg) or a 24-hr transdermal patch (~60 mg/kg) revealed similar organ distribution, including skin. Pigmented tissue, namely, skin and eye showed sustained labeling over 7-10 days, suggesting melanin binding. Subsequently, a 14C-donepezil ADE study in Long-Evans rats comparing seven daily oral doses (10-30 mg/kg) with a seven-day transdermal patch (~40 mg/kg) again revealed similar tissue distributions between the two delivery methods, however, skin not directly exposed to the transdermal patch had less label as compared to both unpigmented and pigmented skin after oral dosing. Similarly, eye concentrations generally had higher label after oral dosing as compared to the transdermal administration. Upon patch removal, the non-viable stratum corneum under the dose-site contained the majority of the label with the underlying skin layers returning to baseline levels within 5 and 7 days for unpigmented and pigmented skin, respectively, illustrating decreased release. The apparent melanin-binding potential of donepezil has not been associated with significant adverse clinical findings, as reported for Aricept®. In conclusion, the comparison between orally administered donepezil and the seven-day transdermal patch in ADE studies provide adequate bridging data with existing rat oral 2-year carcinogenicity data.

**1561** ANTI-ALLERGIC INFLAMMATORY EFFECTS OF PUTRANJIVAIN A: SIGNALING PATHWAY AND ROLE OF NFAT AND NF-κB.

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Mast cells are necessary for the development of allergic reactions, but have been increasingly implicated in innate and acquired immunity, as well as in inflammatory diseases. Activated mast cells, through their effectors and regulatory functions, play a pivotal role in immune responses and the development of chronic inflammation by releasing pro-inflammatory cytokines. Mast cell-mediated allergic inflammation is known to cause many diseases such as asthma, atopic dermatitis, anaphylaxis, and rheumatoid arthritis. Putranjivain A (PTA), isolated from Euphorbia jokilini Bios (Euphorbiaceae), was investigated for its anti-allergic inflammatory activity in mast cell-based in vivo and in vitro models. PTA dose-dependently decreased the gene expression and production of pro-inflammatory cytokines. PTA attenuated activation of NFAT and NF-κB as indicated by inhibition of degradation of IkBα, nuclear translocation of NF-κB, and NF-κB-dependent gene reporter assay via calcium signal. Oral administration of PTA significantly reduced IgE-mediated cutaneous anaphylaxis and expression of histamine H1 receptor in mouse skin. Moreover, PTA dose-dependently inhibited the histamine release induced by PMACI from mast cells. These results suggest PTA attenuates the allergic inflammatory response in activated mast cells by modulating nuclear transactivation of NF-κB and downstream cytokine production, and by controlling histamine release and expression of histamine H1 receptor.

**1562** TOXICITY OF 1-BROMOHEXANE AND ITS CONJUGATION WITH GLUTATHIONE IN MICE.


Immunotoxic effects of 1-bromohexane (1-BH) and its conjugation with glutathione (GSH) were investigated in female BALB/c mice. The animals were treated orally with 1-BH at 500, 1000 and 2000 mg/kg in corn oil once for a dose response study or treated orally with 1-BH at 2000 mg/kg for 6, 12, 24 and 48 hr for a time course study. The treatment of mice with 1-BH increased the serum activities of ALT and AST dose-dependently. The hepatic contents of thio Barbitalus acid reactive substances were significantly increased at 2000 mg/kg of 1-BH from 12 to 24 hr after the treatment. An oral 1-BH significantly suppressed the antibody response to SRBCs and the production of splenic intracellular IL-2 in response to Con A at 2000 mg/kg. The S-bromohexyl GSH were identified in liver by liquid chromatography-electrospray ionization tandem mass spectrometry. The hepatic contents of GSH were maximally decreased 6 hr after the treatment with 1-BH. When the production of conjugates from 1-BH was investigated in livers, the GSH conjugates were also detected maximally 6 hr after the treatment. Our present results suggested that 1-BH could cause hepatotoxicity and immunotoxicity as well as depletion of GSH content, due to the formation of GSH conjugates with 1-BH in female BALB/c mice.

**1563** DONEPEZIL TRANSDERMAL DELIVERY: USE OF ABSORPTION-DISTRIBUTION-EXCRETION (ADE) DATA TO SUCCESSFULLY BRIDGE EXISTING RAT ORAL CARCINOGENICITY DATA.

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The U.S. Environmental Protection Agency’s (U.S. EPA) National Center for Environmental Assessment (NCEA) has compiled a database of key physiological information to improve the consistency and transparency of models that are updated as new data become available. The main goal of this effort is to provide assurance procedures, protocols established to keep the database updated, and exist ing or inclusion. This presentation provides examples of the available data, extracted data from more than 700 peer-reviewed publications. Specific quantitation or inclusion. This presentation provides examples of the available data, extraction or inclusion. This presentation provides examples of the available data, statistical methods used to develop parameter values, implementation of quality assurance procedures, protocols established to keep the database updated, and existing data gaps. The database will be available to the public via NCEA’s Health and Environmental Research Online (HERO) database system, and will be continually updated as new data become available. The main goal of this effort is to provide model developers, users, and peer reviewers a readily accessible database of supporting information to improve the consistency and transparency of models that are used in U.S. EPA’s risk assessments. This work will increase the utility of PBPK models in health risk assessments and will be of great benefit to the scientific and risk assessment communities. Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.
1565 INFLUENCE OF EXPOSURE ROUTE AND DOSAGE REGIMEN ON 1, 1-DICHLOROETHYLENE (DCE) TOXICOKINETICS (TK) AND TOXICITY.

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The objective of this investigation was to elucidate the effects of route of exposure and oral dosage regimen on the TK and hepatoprotective toxicity of DCE. Male Sprague-Dawley rats inhaling 100 or 300 ppm for 2 hours absorbed total doses of 10 and 30 mg DCE/kg, respectively. Other groups of fasted rats received 10 or 30 mg DCE/kg by i.v. injection, gavage (p.o.), or gastric infusion (g.i.) over 2 hours. Serial micro blood samples were taken from the unanesthetized animals and analyzed for DCE content by gas chromatography in order to obtain time-concentration profiles. Serum sorbitol dehydrogenase (SDH) activity was measured as an index of liver damage, while urinary N-acetyl-β-D-glucosaminidase (NAG) and gamma-glutamyltransferase (GGT) activities were monitored as indicators of kidney injury in the high-dose groups. Inhalation resulted in substantially higher peak blood concentrations (Cmax) and area under blood versus concentration time curves (AUC20) than did g.i. of the same dose over the same time-frame, though inhalation AUC∞ values were only modestly higher. Nevertheless, urinary NAG and GGT excretion were much more pronounced following inhalation than g.i. Administration of DCE by gavage also produced much higher Cmax and AUC20 values than did 2-hour g.i., though AUC∞ values were little different. The 30 mg/kg bolus dose produced marked elevation in SDH, though administration of this dose by inhalation and g.i. was only marginally hepatotoxic. These findings demonstrate the TK and target organ toxicity of DCE vary substantially between different exposure routes, as well as oral dosage regimens, making direct extrapolations untenable in health risk assessments. Supported in part by U.S.DOE DE-FCO0-02CH11109.

1566 ARSENIC METHYLATION PHENOTYPE AFFECTS ACCUMULATION AND RETENTION OF ARSENIC IN MICE.


Enzymatically catalyzed methylation of arsenic (As) determines its systemic distribution and retention and its actions as a toxicant and carcinogen. In the mouse, arsenic (+3 oxidation state) methyltransferase (As3mt) catalyzes reactions that convert inorganic arsenic (As) to mono- and dimethylated metabolites. As3mt knockout (KO) mice exhibit a marked, albeit not complete, reduction in formation of methylated metabolites of As. We compared distribution and retention of radioAs in adult female As3mt KO mice and the parental wild type C57BL/6 mice. Each mouse received a daily oral dose of 0.5 mg of As as [3As]-labeled arsenite per kg body weight and was radioassayed immediately before and after dosing to determine As body burden. Body burdens were at steady state after 10 daily doses. Dosing then ceased and As body burdens were measured in an elimination phase, during which body burdens declined to ~50% (As3mt KO) or ~65% (C57BL/6) of steady state levels. Tissues were collected at steady state and after the elimination phase. At steady state in As3mt KO mice, As body burdens were ~20-fold higher than in C57BL/6 mice. At steady state, fractions of As body burden in skin, liver, uterine bladder, kidney, and brain of As3mt KO mice exceeded those in C57BL/6 mice. At the end of the elimination period, phenotypic differences persisted in fractions of the body burden in these tissues. Taken together, these results show that diminished capacity for As methylation in As3mt KO mice affects tissue distribution of orally administered arsenate and the rate of tissue clearance of retained As. These changes increased both tissue contents of As and the integrated exposure of a tissue to As. Altered tissue tropism or retention of As in As3mt KO mice could affect the range of toxic or carcinogenic effects associated with chronic exposure to this metalloid. (This abstract does not reflect U.S. EPA policy.)

1567 DETERMINATION OF TISSUE BLOOD PARTITION COEFFICIENTS FOR NON-VOLATILE HERBICIDES AND FUNGICIDES USING NEGLECTIBLE DEPLETION SOLID PHASE MICROEXTRACTION (NP-SPME).

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Partition coefficients (PC) are parameters used in physiologically-based pharmacokinetic models to estimate the free concentration of chemicals in specific tissues and organs. PC values have been quantified for volatile compounds using air to blood or tissue ratios; however, the same method cannot be applied for non-volatile compounds. The objective of this research project was to develop and apply an analytical method for measuring PCs of non-volatile compounds. Buffered water was used instead of air. Ultra-filtration filters used to separate the blood and tissues from the water were found to significantly retain compounds with Kow ≥ 1. Instead, a negligible depletion solid phase microextraction (nd-SPME) method, not requiring filtration, was implemented. Only the free-phase compounds and not tissue-bound can partition to the SPME fiber coating. PC values were measured for a series of low volatility pesticides with water solubility ranging from 0.07 to 990 mg/L and Kow between 1.9 and 5.5. Tissue-blood PC values were quantified for liver, brain, skin, fat, kidneys and muscle of male Sprague-Dawley rats. Tissue and blood (0.01 to 0.5 mL) were added to 8.5 mL solutions spiked with the chemicals (0.07 to 20 ppm in phosphate buffered saline) and allowed to equilibrate for 4+ hrs at 37°C. Free concentrations were measured (3 to 8 replicates) by inserting a polydimethylsiloxane fiber (PDMS, 100 um thick, 1-10 mm length) directly in the solutions for 1 min. The extracted compounds were desorbed into a gas chromatograph-mass spectrometer for quantification. The method was validated to ensure negligible depletion during extraction. Coefficient of variations for the measured data ranged from 4 to 65% with most of the coefficients (85%) below 35%. (Funded by Syngenta Crop Protection Inc.)

1568 THE TOXICOKINETICS (TK) OF DELTAMETHRIN (DLM) DURING MATURATION OF THE RAT.

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Immature rats are more susceptible than adults to the acute neurotoxicity of pyrethroid insecticides like DLM. A companion TK study (Kim et al., 2009) revealed that brain and brain:fat ratios of the neurotoxicant JT333 were inversely related to age in rats 10 – 90 days old. The current study’s objective was to modify a physiologically-based pharmacokinetic (PBPK) model of DLM for the adult male Sprague-Dawley rat (Mifrazefian et al., 2006), so blood and target organ dosimetry could be predicted during maturation. Age-specific organ weights were estimated with a generalized Merck model. Age-dependent changes in the oxidative and hydrolytic clearance of DLM were modeled, and the summary equations incorporated into the PBPK model. The model’s simulations compared quite favorably with empirical DLM time-courses in plasma, blood, brain and fat for the age-groups evaluated (10, 21, 40 & 90 days old). Clearance increased with age until postnatal day (PND 90). PND 10 pups’ area under the 24-h brain concentration time curve (AUC240) was 3.4-fold higher than that of the PND 90 adults (i.e., similar to the 3.16 PK component of the 10-fold children’s uncertainty factor utilized in pesticide risk assessments). Our maturing rat PBPK model allows for updating with age- and chemical-dependent parameters, so pyrethroid dosimetry can be forecast in young and aged subjects. Supported by U.S. EPA Star Grant 8308000. (This abstract does not represent U.S. EPA policy.)

1569 PLASMA, FAT, AND MILK CONCENTRATION DATA FOR INDOXACARB ESTABLISH BIOEQUIVALENCE OF EXPOSURE IN LACTATING RATS AND OFFSPRING.

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Indoxacarb is a pro-insecticide of the oxadiaziniane family chemical for which metabolism to IN-3733 is required for action as a sodium channel blocker in lepidopteran larvae. Extensive toxicological testing has been performed on its enantiomer (S,R) forms DPX-JW062 (50:50), DPX-MP062 (75:25) and DPX-KN128 (S only). This presentation compares plasma concentration data in lactating female rats, their offspring, and adult non-pregnant Sprague-Dawley rats. Developmental neurotoxicity (DNT) range-finding studies for MP062 and KN128 were conducted by repeated dose oral gavage starting on gestation day (GD) 6. For MP062, maternal dosing (4 mg/kg/day) was GD6 to lactation day (LD) 21, without direct dosing of offspring. For KN128, maternal dosing was GD6-GD12 followed by direct oral gavage dosing of offspring LD11-22 (0, 1.5 and 3 mg/kg/day). Maternal milk, plasma and fat and offspring plasma and fat concentrations were collected on LD11 and LD22, 2-4 hours after dose administration. Plasma and fat samples from male and female (non-pregnant) rats administered JW062 in the diet were collected after 42 days of exposure (5.8 mg/kg/day). Parent chemical and JT333 were analyzed by LC/MS/MS. The key finding from the dietary exposure study was that JT333 in plasma and fat demonstrated steady-state concentrations by 7-14 days of exposure; mean (±SE) JT333 plasma concentration was 303±33 nM. Plasma JT333 in the MP062 DNT study was 212±20 and 202±30 nM, after 28 and 38 days of dosing, respectively. These were remarkably similar to the plasma concentration of 341±84 nM measured in lactating female rats on LD11.
after 27 days of dosing with KN128. Offspring JT333 plasma concentrations were 2-3x greater than JT333 in maternal plasma for MP062 and 1.2-1.3x greater for KN128. These results demonstrate equivalency of maternal plasma exposure and lactational transfer to offspring, and similarity of blood concentrations in pregnant vs non-pregnant rats.

**1570** TOXICOGENETIC DIFFERENCES BETWEEN TWO MAJOR HBCD STEREOISOMERS: EFFECT OF DOSE, TIME, REPEATED EXPOSURE, AND ROUTE.

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Hexabromocyclododecane (HBCD) is a flame retardant added to foam used in building insulation. Global demand for energy-efficient construction is resulting in increasing use of HBCD. This emerging contaminant is manufactured as a mix of 3 stereoisomers (α: (α; 12%), β: (β; 6%), γ: (γ; 82%)), but a shift from the dominant γ to the α in humans and biota has been observed. To aid in predicting health risks, the kinetics of γ and α were investigated. For dose/response (3-100 mg/kg), time course (3 mg/kg up to 14 days), route (3 mg/kg oral or iv) and repeated dose (3 mg/kg/day for 10 days), 60 day old C57BL/6 female mice were treated with a single oral dose (except iv) of 14C-α or γ. Tissues were collected 4 days post exposure. 14C was measured by combustion and LSS, metabolites characterized by TLC and LC-MS/MS. After 14C-γ exposure, disposition was dose independent and linear across all doses with highest levels (9% dose) in liver (0.28), blood (0.08) and fat (0.001). By 1 day, it was rapidly eliminated in urine (23%) and feces (50%) with a 5 day half life. Liver and feces contained metabolites (79-86%) and parent (4-6%). Hydrolysis, de bromination, and stereoisomerization of γ to β and α (11-15%) were identified. Blood, bile and urine contained metabolites after γ or α exposure. Both were well absorbed orally, >95%. After 14C-α exposure, tissue levels were higher than observed with γ and deposition was a function of dose in fat, liver, muscle and skin. Repeated exposure supports bioaccumulation of HBCD. By 1 day, it was eliminated slower in urine (16%) and feces (26-42%) with a 21 day half life. Liver and feces, the parent α predominated (62%), and no stereoisomerism was observed. Dose-dependent decreases in fecal elimination suggest saturation of hepatic enzyme/transporters. We conclude the persistence of γ in mice is low and may explain low levels of γ in biota. Predominance of α in biota is likely due to slower metabolism and lack of stereoisomerism. Absorption does not reflect U.S. EPA, USDA and NCI/NIEHS policy.

**1571** TETRABROMOBIPHENYL A IS NOT NEPHROTOXIC DUE TO ITS TOXICOGENETIC CHARACTERISTICS.


Tetrabromobiphenyl A (TBBPA) is one of the most widely used brominated flame retardants for fire safety of laminates in electronic and electronic equipment. To investigate the nephrotoxic potential of TBBPA and its toxicokinetic profile, single dose and 14-day repeated dose toxicity studies at 200, 500, and 1000 mg/kg were performed in male Sprague-Dawley rats. High dose of TBBPA significantly elevated the renal TBARS content and the activity of superoxide dismutase, and slightly affected the activity of catalase only when it reached the maximum serum concentrations of HBCD not distribute primarily to lipophilic tissues and values were lower (≤50%). Tissue concentrations of HBCD were highest in liver (2.8 PND10; 0.28 PND60), fat (1.8 PND10; 0.001 PND60), brain (0.7 PND10; 0.08 PND60), muscle (0.48 PND10; 0.001 PND60), blood (1.5 PND10; 1.1 PND60), brain (0.8 PND10; 0.6 PND60). In contrast, HBCD γ-derived radioactivity did not distribute primarily to lipophilic tissues and values were lower (≤50%). Tissue concentrations of HBCD γ were highest in liver (2.8 PND10; 0.28 PND60), fat (1.8 PND10; 0.001 PND60), blood (0.7 PND10; 0.08 PND60), muscle (0.48 PND10; 0.001 PND60), brain (0.8 PND10; 0.6 PND60). The nephrotoxic potential of TBBPA and its toxicokinetic profile suggested the presence of glucuronide conjugates, including a major metabolite, the glucuronide of 2,4-dihydrobenzophenone (Benzophenone-1; also BP1). This project was conducted under NIEHS contract N01-ES-75562 (HHSN291200775562C).

**1572** EFFECTS OF RUTAECARPINE ON THE PHARMACOKINETICS OF CAFFEINE IN RATS.

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A possible interaction between rutaecarpine and caffeine was investigated in male Sprague Dawley rats in the present study. Rats were orally administered with 80 mg/kg rutaecarpine for three consecutive days. Twenty four hr after the last treat-
veloping animals to adverse effects of HBCD. This abstract does not reflect U.S. EPA, USDA, and NCI/NIEHS policies. Supported by NIH Training Grant T32-ES07126 and U.S. EPA CR833237.

1575 BUPRENORPHINE MODULATES METHAMPHETAMINE-INDUCED EXTRACELLULAR DOPAMINE RELEASE IN THE RAT CAUDATE-PUTAMEN.


The instrumental use of a stimulant, methamphetamine (METH), for functional enhancement often leads to dependency. Oxidative stress mediates to a large extent METH-evoked neurotoxicity. Clinical trials are being conducted to identify pharmacotherapeutic adjuncts to behavioral therapy in the treatment of METH dependence. Efficacy of the adjunctive medication with opioid compound, buprenorphine (BUP), has been demonstrated in the treatment of opiate and cocaine dependence. Here, we examined the effects of a dopaminergic response to a combined METH/BUP treatment in the rat striatum. In vivo microdialysis and high performance liquid chromatography with electrochemical detection (HPLC-EC) were used to measure baseline and METH-stimulated striatal dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) levels in adult male Sprague-Dawley rats. Animals received either 2 mg/kg METH, i.p. or BUP at 0.01 or 10 mg/kg, s.c. alone 10 min before 2 mg/kg METH. Dialysates were assayed every 20 min for 300 min following METH administration. Compared with baseline (100%), a sharp DA increase to 1090 ± 175% was observed after METH treatment. While treatment with 0.01 and 10 mg/kg BUP alone did not alter DA levels, rats treated with METH and 0.01 or 10 mg/kg BUP exhibited an extracellular increase in DA of 439 ± 90% and 639 ± 55%, respectively. The duration of the DA response and the area under the curve were attenuated as well. Although DOPAC levels didn't change from baseline following 0.01 mg/kg BUP (BUP0.01), the concentration of DOPAC was significantly lower after the combined METH/BUP0.01 treatment throughout the experiment. On the other hand, extracellular DOPAC levels increased (p < 0.05) within 140 min following treatment with 10 mg/kg BUP (BUP10). METH/BUP10 treatment induced a decrease in the DOPAC levels that lasted for 150 min. Data indicate that BUP modulates the DA response to a METH challenge and may suggest effectiveness of low-dose BUP treatment in METH addiction.

1576 TOXICOKINETICS OF RESERVATROL IN MALE, FEMALE, PREGNANT, AND LACTATING WISTAR HAN RATS.

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Resveratrol, a polyphenol found in plants such as grapes and peanuts, has been reported to have antioxidant and cardioprotective effects. Widespread public exposure to resveratrol in diet or supplements has raised concern about potential toxicity. This study was conducted to measure resveratrol in plasma, fetuses and pups at doses proposed for toxicity testing in rats. A single dose of trans-resveratrol was administered p.o. (78 and 1250 mg/kg) to male and female Wistar Han rats. Resveratrol was also administered daily to pregnant Wistar Han rats from gestation day (gd) 11 – 18, or gd 18 - postnatal day (pnd) 3 (78 and 1250 mg/kg/day). Blood, fetuses and pups were collected at 15, 30, 60, 90 min, 4 and 8 hr following the last dose from one rat or dam per timepoint. Resveratrol in adult plasma was measured by UPLC-PDA, and in fetal or pup homogenate by LC-MS/MS for non-compartmental pharmacokinetic analysis. Plasma concentrations ranged from (LOQ (10.2 ng/mL) - 1340 ng/mL). The elimination half-life (t1/2) of resveratrol in male rats was 323 and 395 min at the low and high dose respectively, compared with 168 and 609 min in female rats. AUC increased in a less than dose proportionate manner in male rats. In pregnant rats (gd 11-18), t1/2 was 88 min at the low dose, and 1210 min at the high dose. The increase in AUC was greater than proportional to dose, implying saturation of metabolism. In pregnant/lactating rats (gd 18-pnd 3), t1/2 was 37 min at the low dose, and 148 min after the high dose. The highest plasma concentrations were observed in the high dose pregnant/lactating group (1340 ng/mL). At gd 18, AUC was similar in maternal plasma and fetal tissue, but on pnd 3, AUC in pups was 6-7 fold higher than in maternal plasma. This study established plasma resveratrol concentrations expected during toxicity testing in pregnant and lactating rats, and indicated that resveratrol is transferred to the fetus in pregnancy and to the pup by lactation.

1577 PRECLINICAL SAFETY ASSESSMENT OF A NEW ANTITHROMBOTIC DRUG, THE NANOBODY® ALX-0081.

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Ablynx develops antibody-derived therapeutic proteins, Nanobodies®, for the use in patients affected by various diseases including cardiovascular, bone and inflammatory. They are based on the smallest functional fragments of heavy chain antibody bodies, which occur naturally in the Camelid family. ALX-0081, a bivalent Nanobody® manufactured in E.coli, targets the platelet adhesive von Willebrand Factor (vWF). It is formulated for i.v. (drug product: ALX-0081) or s.c. administration (drug product: ALX-0081). ALX-0081 and ALX-0081 are currently in clinical development Phil and Phil respectively. ALX-0081 specifically blocks interaction of the A1-domain in regular sized and ultra-large vWF multimer with the platelet receptor GPIb-IX-V. Therefore, this compound could be a powerful inhibitor of the pathophysiology of thrombotic events in several diseases e.g. ACS and TTP. Cynomolgus monkey and guinea pig were identified as the appropriate species for safety assessment. This was confirmed via tissue cross-reactivity staining, in vitro binding/competition and efficacy assays and in vivo PK/PD measurements. The toxicology program includes single dose and repeated dose toxicity studies in both species and an embryo-fetal developmental study in guinea pig. Safety pharmacology, fertility testing and local tolerance are incorporated in the repeated dose studies. Maximum target concentrations were evaluated in the design and dosing schemes guided by a mechanistic PK model describing clearance mechanisms and drug-target relationships of ALX-0081. ALX-0081 exhibits no sign of adverse effects in both species so far due to (i) a unique PK behavior where only drug bound to the target vWF is retained in circulation and excess drug is rapidly eliminated, (ii) a lack of secondary pharmacological effects and (iii) a modular design highly specific for pathological events. The sole treatment-related effect noted was a decrease in FVIII and vWF, which can be attributed to the pharmacology of ALX-0081 as vWF serves as a carrier for FVIII.

1578 TOXICOKINETICS OF PERFLUOROOCATANIC ACID (PFOA) AND PERFLUOROOCTANE SULFONATE (PFOS) USING MALE SPRAGUE-DAWLEY RATS AND AUTOMATED DOSING/SAMPLE COLLECTION INSTRUMENTS (EMPS/CULEX).

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PFOA and PFOS are considered global contaminants due to detection in humans and wildlife in various geographical locations. This study determined plasma concentrations for estimation of TK parameters of PFOA and PFOS and examined the effect of co-administration of PFOA and PFOS. Three rats were given a single IV administration of either 6.0 mg/kg PFOA, 2.0 mg/kg PFOS, or 6.0 mg/kg PFOA co-mixed with 2.0 mg/kg PFOS in 2% Tween 80 in deionized water, delivered by the automated dosing instrument (Empus). The dosing volume was 4 mL/kg. Blood samples were collected by the automated sampling collection instrument (Culex). The plasma samples were separated and analyzed by LC-MS/MS. The use of the Empus/Culex significantly reduced the number of animals on study by allowing sample collections from each rat to construct a complete TK profile. Both PFOA and PFOS plasma concentration time profiles for the rats exhibited a biphasic decline, which supported the use of a two-compartment model with first order elimination. PFOA and PFOS exhibited similar TK parameter values when considering variability associated with group mean values. PFOA and PFOS had long elimination phases. The beta half-life values for PFOA were 6.89 and 5.08 days and for PFOS were 5.74 and 2.22 days, alone and co-mixed, respectively. Whether PFOA and PFOS were administered alone or together the TK parameter estimates did not substantially change, thereby demonstrating the feasibility of conducting TK studies on PFOA and PFOS as a co-mixture. The results of this study were used to design a preliminary toxicokinetic study in order to correlate toxic effects with systemic availability and to evaluate the feasibility of this automated approach for future studies. [Supported by NIH, N01-ES-55551]
1579 CHARACTERIZATION OF THE EFFECT OF MULTIROUTE EXPOSURE ON THE INTERNAL DOSE OF 2, 2, 4-TRIMETHYLPENTANE (TMP) IN THE RAT.

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Volatile organic compounds (VOCs) in drinking water may be absorbed not only by the oral route but also by the dermal and inhalation routes. The aim of this study was to characterize, in an animal model, the contribution of multiroute exposure to the internal dose of 2,2,4-trimethylpentane (isooctane; TMP). Initial experiments showed that absorption of TMP by the dermal route is insignificant when compared to the other two routes. Consequently, groups of 5 adult male Sprague-Dawley rats were either given a single dose of TMP by gavage (40 or 163 mg/kg; ORA) or exposed by inhalation (INH) to 300 or 1200 ppm during 2 hr. Additionally, groups of rats (n=5) were exposed to TMP by the two routes concurrently to the high doses (ORA: 163 mg/kg and INH: 1200 ppm) or to the low doses (ORA: 40 mg/kg and INH: 300 ppm) investigated in the single route study. Blood samples (50-100 μl) were collected for up to 6 hr after treatment, to compare kinetics following single and multiroute exposures. The blood concentrations (mean ± sd) measured after multiroute exposure to the low doses of TMP were 1.8 ± 0.2 mg/L and 0.49 ± 0.04 mg/L at 2 hr and 4.5 hr post-exposure. These levels were similar (i.e., 1.0 – 1.2 fold) to the sum of the blood levels obtained after administration of TMP by each route: 1.7 and 0.4 mg/L after 2 hr and 4.5 hr. Similarly, experimental results for multiroute exposure to the high doses (7.4 ± 1.0 mg/L and 2.0 ± 0.2 mg/L at 2 hr and 4.5 hr post-exposure) were comparable to the predictions based on additivity of results for ORA and INH routes (6.9 and 1.9 mg/L after 2 hr and 4.5 hr). For both the low and the high doses, the experimental AUCs for multiroute exposures (11 and 2.4 h·mg/L) were equal to the values predicted (11 and 2.4 h·mg/L) based on results for individual routes. These data suggest that, at the doses investigated in this study, the internal dose of TMP associated with multiroute exposures can be predicted by adding the internal dose for individual routes (Supported by NSERC and ExxonMobil).

1582 PHARMACOKINETICS AND DISTRIBUTION OF SB 9002-1 PRO-DRUG FOR HEPATITIS TREATMENT.


SB 9002-1 is a prodrug of SB 9000, a novel di-nucleotide anti viral agent with activity against hepatitis B virus (HBV). Orally administered SB 9002-1 significantly reduces the HBV DNA in the liver as shown by Southern blot hybridization and quantitative PCR. The current studies were undertaken to evaluate pharmacokinetic and tissue distribution of SB 9002-1, which was administered to male and female Sprague-Dawley rats, jugular vein catheterized, at 10 mg/kg, iv, and 10 and 50 mg/kg po. SB 9002-1 was readily converted to its active form, SB 9000 by plasma esterases, and no parent drug was detected in plasma from either iv or po treated rats. Following iv administration, SB 9000 had a t1/2 of ~1 hr and clearance of >6000 ml/hr/kg in plasma. Neither SB 9002-1 nor SB 9000 was quantifiable at any time point in plasma from the po group. To evaluate the tissue distribution of this drug, 158S-SB 9002-1 (120 mCi/mg; 84.9 mCi/mmol) was synthesized and administered to rats, 10 mg/kg, iv and po. Total radioactivity was determined in excrta, major tissues, and the carcass. Radioactivity was readily detected in plasma after both iv and po routes up to the last time point collected at 24 hr. Radioactivity concentrations in the liver and the ratio of liver to plasma concentration was as high as 3.9 (iv route) and 2.9 (po route). At the T max for iv and po routes, ~2.5% and ~0.7% of the dose, respectively, were present in the liver. Other tissues—kidney, brain, lung, spleen, heart contained minor amounts of radioactivity. The primary route of excretion of 35S from SB 9002-1 after iv administration was in the feces with ~60% of the dose in 24 hr. After po administration, radioactivity was excreted about equally in urine and feces, ~35% in each in 24 hr. (Supported by NIAID Contract N01 AI-60011 and U01 GM 058270, R01, PI).

1583 ALLOMETRIC SCALING TO PREDICT CLEARANCE (CL) OF A RECOMBINANT PROTEIN (ENB-0040) IN INFANTS AND ADULTS.

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ENB-0040 is a soluble form of tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) developed by Enobia Pharma for the treatment of hypophosphatasia, a rare metabolic bone disease. The objective of this study was to estimate PBPK parameters and pharmacokinetic properties of ENB-0040 in Weanling Sprague-Dawley rats under varying dosing scenarios. Thus, single and multiple IV and SC dosing scenarios in humans were simulated according to this two-compartment model in infants (5 kg) and adults (70 kg). The human dose of ENB-0040 for subcutaneous (SC) and intravenous (IV) administration was evaluated by developing a PK model based on animal data where PK parameters were extrapolated to humans according to body weight (BW). This PK model was used to estimate exposure in humans under various dosing scenarios. Thus, single and multiple IV and SC dosing scenarios in humans were simulated according to this two-compartment model in WinNonlin®. The simulation of ENB-0040 concentrations following IV dosing was performed using extrapolated systemic CL, clearance of distribution (CL/V), volume of distribution in central compartment (Vr) and the volume of distribution in peripheral compartment (Vp) for typical subjects with body weight values of 5, 30 and 70 kg. The same parameters were also used for SC dosing simulations with an appropriate correction for bioavailability and rate of absorption. The predicted CL values in infants (5 kg) and adults (70 kg) were 0.035 and 0.4 L/h, respectively. This approach was validated using data from the first in human clinical trial, where
ionic liquids (ILs) are a class of salts often referred to "green chemicals". They have been shown to be used as a new source of solvents and for many other applications. It is expected to be used as a new source of solvents and for many other applications.

**1584 CHARACTERIZATION OF THE TRANSPORT AND INHIBITORY EFFECTS OF N-BUTYLPYRIDINUM CHLORIDE AND ITS STRUCTURALLY RELATED IONIC LIQUIDS TO OCT1/2.**

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Perfluorohexanesulfonate (PFHxS) is widely distributed in humans and wildlife. The geometric mean half-life of serum elimination of PFHxS in humans has been estimated to be 7.3 years (95% CI 5.8-9.2 years). We undertook a series of studies to establish pharmacokinetic parameters in non-human species. Male (M) and female (F) monkeys were given a single intravenous (IV) dose of K-PFHxS and serum and urine PFHxS concentrations were followed for 171 days. M and F rats were given single oral doses of 10 mg/kg K-PFHxS and urine and feces were collected over 96 hours and serum and liver collected at 96 hours. Jugal-cannulated M and F rats were given IV or oral single 10 mg/kg doses of K-PFHxS and serum concentrations of PFHxS were followed for 24 hours. M and F rats were given a single IV dose of 10 mg/kg, and serum, feces, and urine were collected weekly for 10 weeks. All PFHxS analyses utilized LC-MS/MS methods. Pharmacokinetic parameters were determined by WinNonlin® software. Volumes of distribution indicated predominant extracellular distribution. Mean serum elimination half-lives were 141 and 87 days for M and F monkeys and ca. 30 and 1.5 days for M and F rats.

**1585 COMPARATIVE PHARMACOKINETICS OF PERFLUOROHEXANESULFONATE (PFHxS) IN RATS AND MONKEYS.**


**1586 THE EFFECT OF COLESEVEMAL HYDROCHLORIDE ON THE ELIMINATION OF POTASSIUM PERFLUOROOCTANOATE (PFOA) FROM SERUM IN MALE AND FEMALE Cynomolgus MONKEYS.**

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Perfluorooctanoate (PFOA) is widely distributed in humans and wildlife. Due to its slow elimination in humans (serum geometric mean half-life (T1/2)=3.5 yrs), this study was to evaluate whether colesevelam hydrochloride (colesevelam HCl, an anion-exchange resin compound that lowers lipids by impeding bile acid absorption in the intestine), can affect the elimination of PFOA from serum in cynomolgus monkeys. On study day (SD) 0, monkeys (N=6/sex/group, ~ 2 to 5 yrs old) received a single intravenous dose of K-PFOA (10 mg/kg baseline). On SD 21-27 and 56-83, daily oral doses of placebo (water) were given to Group 1 monkeys while Group 2 monkeys received placebo on SD 21-55. On SD 28-55, daily oral doses of colesevelam HCl (250 mg/kg/d) were given. Group 1 monkeys while Group 2 monkeys received colesevelam HCl on SD 56-83. Serum samples were collected and analyzed for PFOA concentrations using LC/MS-MS from each monkey prior to dosing (0 hr), on SD 0 (0.5 hr post iv dose), 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84. All monkeys survived until the end of the study. There were no clinically significantly abnormal observations noted and body weights were comparable between dose groups in each sex for the duration of the study. In either male (M) or female (F) monkeys, there was no apparent difference in mean serum PFOA levels between Group 1 (given colesevelam HCl on SD 21-55) and Group 2 (given colesevelam HCl on SD 56-83). PK parameters indicated similar mean overall serum half-life (T1/2) and AUC values of PFOA in M monkeys in Group 1 (T1/2=18.8 days, AUC=1965 hrdg/mL) and Group 2 (T1/2=17.0 days, AUC=1716 hrdg/mL); as well as in F monkeys in Group 1 (T1/2=26.3 days, AUC=2869 hrdg/mL) and Group 2 (T1/2=28.4 days, AUC=2192 hrdg/mL). Therefore, administration of colesevelam HCl had no therapeutically significant impact on serum PFOA levels, serum PFOA half-life, or serum PFOA AUC levels within the limits of the study design.

**1587 ANALYTICAL METHOD VALIDATION OF N-BUTYL GLICYDYL ETHER IN CORN OIL.**

J. W. Algieri1,  D. M. Logan1,  V. F. Ault1,  L. O. Beverley1,  A. Kastori1,  B. M. O’Brien1,  J. J. Butenhoff1,  R. K. Harris1,  B. Jayaram1 and  C. S. Smith1.

The compound n-Butyl glycidyl ether (nBGE) is a high production volume chemical used in the production of epoxy resins. Epoxy resins are used in many commercial industries such as construction, electronics manufacturing and coating applications. The ubiquitous presence of epoxy resins, and their components, in these industries has lead to concern for worker exposure. For this reason, nBGE has been selected for toxicological evaluation by the National Toxicology Program (NTP). An analytical method was developed and validated for the analysis of nBGE in corn oil to cover a dose formulation range of ~4.0 to ~100 mg/mL. Standards were prepared by adding nBGE and the internal standard (IS), 1-undecene, to corn oil and diluting the mixture with THF. The standards were analyzed using gas chromatography (GC) with flame ionization detection (FID) equipped with a Restek RTX-1701 (30 m x 0.53 mm I.D., 1.5-μm film thickness) column. The oven program was 40 °C (1-min hold) to 140 °C at 5 °C per minute and then to 240 °C at 20 °C/min. The retention time was 12.4 min for nBGE and 13.0 min for the IS. The method validation was linear (r2=0.9999), accurate (from -0.8 to 0.9% relative error) and precise (9% RSD on average). Following method validation, formulation stability study and a 3-hour simulated dosing study were performed at 10 mg/mL nBGE in corn oil. The 42-day dose formulations were stored under ambient (25 °C) and refrigerated (5 °C) conditions. When compared to Day 0 values, the results indicated losses ≤0.6% under ambient conditions and ≤1.1% under refrigeration. The 3-hour simulated dosing study indicated that stored in clear serum vials exposed to air and light under ambient (25 °C) temperature. When compared to Day 0, the results indicated losses ≤0.6%. A high dose formulation of ~410 mg/mL nBGE in corn oil was evaluated for homogeneity and was shown to be homogeneous based on an observed RSD of 0.7% for n=5 samples.

**1588 ANALYTICAL METHOD VALIDATION FOR THE QUANTITATION OF SIX NITROSAMINES IN NTP-2000 RODENT FEED.**

P. J. Schelber1,  A. D. Ammenhauser1,  J. L. Cookinham1,  J. W. Algieri1,  R. K. Harris1,  B. Jayaram1 and  C. S. Smith1.

Nitrosamines, a class of compounds identified as potentially carcinogenic, are found in beverages, nitrate cured foods and may be formed during cooking or food processing. The presence of nitrosamines in specialized rodent diets used for toxicology studies can potentially confound the interpretation of data from a toxicological study. Thus, is important to demonstrate that rodent diets used in these studies are low in nitrosamines. To this end the National Toxicology Program has sponsored the development and validation of a chromatographic method to quantitate six nitrosamines (N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodibutylamine, N-nitrosopiperidine, N-nitrosopyrrolidine, and N-nitrosonornicotine) in NTP-2000 feed. Matrix standards covering a concentration range of
-10 to -50 ng/g were prepared by spiking the six nitrosamines and the internal standard into feed. The matrix standards were extracted with methanol and analyzed using gas chromatography with an Agilent 235 nitrogen chemiluminescence detector with a dual plasma and controlled by the National Toxicology Program (NTP), which is a flame retardant added to flexible polyurethane foam used in home furnishings. An analytical method was developed and validated for the analysis of TCPP, with a 0.5% aqueous methylcellulose formulation to cover a dose formulation range of -0.79 to -31.84 mg/mL. Aliquots of the formulation were prepared for analysis by adding the internal standard (IS), isodrine, and diluting the mixture with methanol. Samples were analyzed using gas chromatography (GC) with flame ionization detection (FID) equipped with an F and W Scientific DB-5 (30 m x 0.32 mm I.D., 1.0 μm film thickness) column. The oven program was 160°C (5 min hold) to 180°C (3 min hold) at 1.0°C/min. The retention times for the three major isomers of TCPP were 15.0, 15.6, and 16.1 min with 27.1 min for the IS. The method was linear (correlation coefficient ≥ 0.9999), precise (≤ 1.5% RSD), and accurate (RE from -9.0% to 1.6%). TCPP recovery for the spiked vehicle standards was 98.7 ± 3.2% when compared to the expected concentration and 99.1 ± 1.2% when compared to solvent standards. A 42-day dose formulation stability study and a 3-hr simulated dosing study were performed at 1.56 mg/mL and in 0.5% aqueous methylcellulose formulation. The 42-day stability samples were stable for 7 days as defined less than 10% loss, when stored under ambient (25°C) and refrigerated (5°C) conditions. The 3-hr simulated dosing formulations were stored under ambient conditions in clear glass vials, exposed to light and air. The results showed a 3-hr simulated dosing study indicated a 6.4% increase in TCPP concentration when compared to day 0 values. Low and high dose formulations were also analyzed for homogeneity.

Activity of superoxide dismutases (SOD) in liver and uterus (total SOD, CuZnSOD and MnSOD). As expected, the positive control, ciprofibrate, increased liver weight and peroxisomal acyl Co-A oxidase activity in the liver and altered antioxidant enzyme activities in the uterus and liver. Levels of N-EtFOSE and its metabolites decreased in the order of perfluorooctanesulfonate (PFOS) >> perfluorooctanesulfonamide - N-ethyl perfluorooctanesulfonamidocacetate >> perfluorooctanesulfonamidoethanol - N-EtFOSE, which is in agreement with published in vitro metabolism pathways. Overall, this study demonstrates the biotransformation of N-EtFOSE to PFOS in rats that is accompanied by N-EtFOSE-induced alterations in antioxidant enzyme activity.

Sulfonyl fluoride (SO$_2$F) is a structural and post-harvest fumigant and is a key alternative to methyl bromide which is scheduled for phase-out by the Montreal Protocol. Perinatal pharmacokinetics (PK) of inhaled SO$_2$F$_2$ and its hydrolysis products, fluorosulfate (FSO$_3$) and fluoride (F) were examined at the end of gestation on gestation day (GD) 20, during lactation on lactation day (LD) 10, and following direct gavage exposure of pups with FSO$_3$ and F on postnatal day (PND) 10. Dams were exposed to 0, 5, 30 or 150 ppm SO$_2$F$_2$, 6 h/day by inhalation, from GD 6-20 and LD 5-10; PND 10 pups were given a single gavage dose of 4, 20 or 40 μg each of FSO$_3$ and F in rat milk vehicle and blood samples were collected 1, 3 and 6 h post-dosing. The perinatal PK of SO$_2$F$_2$ in maternal and young rats shares many common features with the previously described PK of SO$_2$F$_2$ in adult male F344 rats. Similar to the results from adult male PK studies, parent SO$_2$F$_2$ was not detected in any maternal or fetal blood samples following exposures ≤ 150 ppm SO$_2$F$_2$ for 6 h and pregnant dams exhibited rapid, first-order elimination of FSO$_3$ and F at all concentrations of SO$_2$F$_2$. During gestation and lactation, fetal and pup plasma levels of FSO$_3$ and F were markedly lower (< 20%) than dam peak plasma FSO$_3$ and F levels, despite 2-4X higher levels of FSO$_3$ and F in milk relative to maternal plasma. Both dams and pups rapidly eliminated FSO$_3$ and F. The perinatal PK data at the 5 ppm exposure concentration, which is most relevant for potential human exposure to SO$_2$F$_2$, clearly demonstrate that there is no evidence of significant internal doses of SO$_2$F$_2$, FSO$_3$ or F to either fetuses or pups following in utero or lactational exposure. Overall, these data also indicate a lower degree of concern for potential pre- and postnatal effects in human infants and children because the metabolic profile for SO$_2$F$_2$ indicates lower internal dose of the active moiety (F) in young as compared to adults.

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Sulfonyl fluoride (SO$_2$F) is a structural and post-harvest fumigant and is a key alternative to methyl bromide which is scheduled for phase-out by the Montreal Protocol. Juvenile pharmacokinetics (PK) of inhaled SO$_2$F$_2$, and its hydrolysis products, fluorosulfate (FSO$_3$) and fluoride (F) were examined in blood/plasma from weanling male rats following a 4-hour inhalation exposure to 0, 3, 30 or 300 ppm on postnatal day (PND) 22. SO$_2$F$_2$ PK in weanling male rats shared many common features with the previously described PK of SO$_2$F$_2$ in adult male F344 rats. Similar to the results from adult male PK studies, parent SO$_2$F$_2$ was not detected in any maternal or fetal blood samples following exposures ≤ 150 ppm SO$_2$F$_2$ for 6 h and pregnant dams exhibited rapid, first-order elimination of FSO$_3$ and F at all concentrations of SO$_2$F$_2$. During gestation and lactation, fetal and pup plasma levels of FSO$_3$ and F were markedly lower (< 20%) than dam peak plasma FSO$_3$ and F levels, despite 2-4X higher levels of FSO$_3$ and F in milk relative to maternal plasma. Both dams and pups rapidly eliminated FSO$_3$ and F. The perinatal PK data at the 5 ppm exposure concentration, which is most relevant for potential human exposure to SO$_2$F$_2$, clearly demonstrate that there is no evidence of significant internal doses of SO$_2$F$_2$, FSO$_3$ or F to either fetuses or pups following in utero or lactational exposure. Overall, these data also indicate a lower degree of concern for potential pre- and postnatal effects in human infants and children because the metabolic profile for SO$_2$F$_2$ indicates lower internal dose of the active moiety (F) in young as compared to adults.
which is most relevant for potential human SO$_2$F$_2$ exposures, clearly demonstrate that there is no evidence of significant internal doses of SO$_2$F$_2$, FSO$_3$, or F following inhalation exposure. Overall, these data indicate a lower degree of concern for potential effects in infants and children, because the metabolic profile for SO$_2$F$_2$ indicates lower internal dose of the active moiety (F) in young as compared to adults.

1593 THE EFFECT OF INCREASING DMSO concentrations on the pharmacokinetics of orally dosed reserpine in male sprague-dawley rats.


Exploratory pharmacokinetic (PK) studies are often performed with formulations containing solvents unlikely to be included in the formulation administered during the later stages of preclinical development. One such solvent is dimethyl sulfoxide (DMSO), which has been reported to possess pleiotropic effects including enhanced membrane transport, diuresis, and altered effectiveness of co-administered drugs. As DMSO alters membrane transport and enhances diuresis, its inclusion as a formulation excipient may alter the PK parameters, particularly bioavailability and clearance, of the test article being studied. Thus, researchers risk making drug candidate selection decisions with PK data that may be altered when DMSO is removed from the formulation. To examine the effect of DMSO concentration on the PK parameters of a given test article, reserpine was selected on the basis of several intrinsic characteristics (i.e., moderate oral bioavailability, P-glycoprotein inhibition, and a predominantly fecal excretion route). Reserpine was prepared as an oral formulation by solubilization in 100% DMSO at three concentrations, or it was left as a bulk powder. The reserpine powder or reserpine/DMSO stock solutions were added to quadruplicate sets of a 0.5% methylcellulose solution to prepare 4 separate dosing formulations with a constant dose concentration of 0.3 mg/mL and final DMSO concentrations of 0, 5, 15, and 25%, respectively. Each of the formulations was orally administered at 3 mg/kg to 4 separate groups of male Sprague Dawley rats (n=3/group). Following administration, blood, urine and fecal samples were collected at 0.5, 1, 2, 4, and 6 hours post dosing, and analyzed for test article concentration by LC/MS/MS. The resulting bioanalytical data was used to generate formulation-specific PK parameters. These profiles were compared to each other and the published PK data for reserpine (50% bioavailability, 62% protein binding, 4.5 hr half-life, 60% fecal excretion, 10% urine excretion) to determine the impact of DMSO concentration on reserpine’s PK parameters.

1594 PHARMACOKINETICS AND BIOAVAILABILITY TESTING OF CIPROFLOXACIN IN CANNULATED RATS.

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The purpose of this study was to examine drug exposure of the antibiotic ciprofloxacin in male and female Sprague Dawley rats following oral and intravenous administration. Pharmacokinetic modeling and determination of bioavailability were conducted following single dose treatment and subsequent measurements of Ciprofloxacin levels in plasma. Dose levels studied were 250 mg/kg oral and 25 mg/kg intravenous (IV). No statistical difference in exposure following oral gavage administration was observed between male and female rats. The AUC0-∞ following an oral dose of 250 mg/kg was 6097 ± 5139 ng·hr/mL in males and 5111 ± 1245 ng·hr/mL in females (p = 0.648). However, there was a statistical difference in exposure between sexes to administration of IV ciprofloxacin with male rats displaying a higher exposure than female rats. The AUC0-∞ following an IV dose of 25 mg/kg was 10164 ± 607.7 ng·hr/mL in males and 7697 ± 1054 ng·hr/mL in females (p = 0.0391). Due to the low numbers of animals and variability in the results, more testing would be needed to confirm this sex-related difference in AUC0-∞. The maximum observed concentration following IV dosing was 7342 ± 2566 and 6297 ± 2573 ng/mL for males and females, respectively. Following oral administration of ciprofloxacin, the maximum plasma concentration was 1278 ± 780.6 and 1051 ± 193.5 ng/mL for male and female rats, respectively. There was little variability in the Cmax of rats, with the exception of one male rat that had over twice the Cmax of the other two animals in the male gavage group, which is not completely unexpected for oral administration. The bioavailability of ciprofloxacin was very low in both male and female rats (6.0% in males; 6.0% in females). The bioavailability between humans and rats differs dramatically. In conclusion, the rat is an adequate model for testing the efficacy of ciprofloxacin following exposure to pathogens; however, the low bioavailability indicates oral administration may be problematic.

1595 DETERMINATION OF HEPATOCELLULAR VOLUME IN RAT AND HUMAN SANDWICH-CULTURED HEPATOCYTES.


Intracellular mass data using B-CLEAR® technology in sandwich-cultured hepatocytes (SCH) has been used to determine the potential for drug interactions and drug-induced liver injury. Intracellular mass data is not as informative as drug concentration within the hepatocyte, since the kinetics of drug transport, metabolism and induction are a function of concentration and not mass. The aim of the present study was to determine the hepatocellular volume of rat and human SCH. 3-O-Methyl-D-glucose (3MG), whose transport is governed by facilitated diffusion, was used to estimate intracellular volume, under the assumption that the intracellular and extracellular concentrations of 3MG were equal when equilibrium was established. Hepatocellular volume was determined as the ratio between intracellular 3MG mass and 3MG medium concentrations. 3MG accumulation in rat SCH was comparable at 10, 30, and 60 minutes, suggesting that equilibrium of 3MG between hepatocytes and incubation medium was established within 10 minutes. Subsequent studies (0.01 – 1 mM 3MG; 30-min incubation) demonstrated that the hepatocellular volume of rat SCH was not statistically different between 6-well and 24-well (6.73 ± 1.25 vs. 6.19 ± 0.676 pl/hepatocyte; n=5) culture formats. Without further incubation extensively, the ratio of the equilibrating population (p) in rat SCH was divided by hepatocellular volume, TGZ and TS hepatocellular concentrations were estimated to be 7.22-47.7 μM and 132-222 μM, respectively. This was much higher than the previously-reported IC50 of TGZ (3.9 μM) and TS (0.4-6.6 μM) for the bile salt export pump, supporting the potential for TGZ-mediated hepatic injury due to increased hepatic bile acid concentrations. In 24-well human SCH, the hepatocellular volume was determined to be 6.03 pl/hepatocyte (n=1). In conclusion, the hepatocellular concentration of test compound, obtained by utilizing hepatocellular volume, may be a better parameter than intracellular mass of compound when evaluating the potential for drug-mediated hepatotoxicity, drug-transporter interactions, as well as for transporter induction potential.

1596 TRANSPORTER EXPRESSION IN RAT NASAL OLFACTORY AND RESPIRATORY EPITHELIA.

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It has been known for some time that agents, ranging from pathogens, to pharmaceuticals, to metals, that are administered into the nasal cavity can be translocated to both the brain and the bloodstream, but the precise mechanisms by which this occurs has not been studied extensively. Due to the extensive use of the intranasal route for pharmaceutical administration, we evaluated the expression of several transporters in rat nasal respiratory and olfactory epithelia, and in olfactory bulb (OB), using the branched DNA signal amplification assay. Expression of Oatp3, an organic anion transporter that transports thyroid hormone and mediates the biliary excretion of bile acid conjugates, was >50-fold higher in both olfactory and nasal respiratory epithelia than in OB, liver, or kidney. Similarly, Mrp4 expression was considerably higher in both nasal epithelia than in OB or liver. Conversely, Mrp1, Ent1, and Ent2 expression was highest in OB compared to the other tissues. Oct2 expression was found to be comparable in olfactory epithelium to that in the kidney. Finally, OctN1 expression was higher in nasal respiratory epithelium than in the other tissues, with comparable, but lower, expression in olfactory epithelium, OB, and kidney. These results may explain, in part, the observations of other investigators, demonstrating the efficient delivery of agents such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DCT) and dopamine into the brain following intranasal administration. A comprehensive understanding of the transporters located in nasal epithelia may aid in design of drugs or drug formulations for administration via the intranasal route.

1597 ASSESSING SUSCEPTIBILITY TO DICLOFENAC TOXICITY IN MRP3-NULL MICE.

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The diclofenac metabolite, diclofenac-1-O-acetyl glucuronide (DG), is a reactive species that covalently binds to cellular proteins in liver and small intestine. These protein adducts can alter protein function and/or elicit an immunogenic response.
1598  THE INTERACTION OF PLACENTAL EFFLUX TRANSPORTERS WITH XENOBIOTICS.

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The placenta plays an important role in normal foetal development, providing the conduit for nutrient and gaseous transport, removal of waste materials and a protective barrier for the foetus against exposure to exogenous agents. Active efflux transporter proteins, including P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) are known to be expressed in the placenta, and are thought to contribute to the barrier-like properties of this tissue. The aim of this project was to explore assays which may be useful in investigating the interaction of placental efflux transporters with xenobiotics. Two functional assays were evaluated, one ex vivo and one in vitro. P-gp function in fresh human placental fragments was assessed by measuring [3H]Taxol (a P-gp substrate) accumulation over time. There was a time dependent increase in [3H]Taxol accumulation in all placentae studied which was differentially sensitive to verapamil (a P-gp inhibitor). In the in vitro model, using P-gp-expressing JA rat placental choriocarcinoma cells (which mimic the trophoblast layer of the placenta), confirmed active extrusion of the P-gp substrate calcein acetoxymethyl (AM). This was inhibited by verapamil, demonstrating in vitro functional activity. The in vitro system was further characterised to assess the ability of a number of therapeutic agents (known to be P-gp substrates), to modulate P-gp activity. These compounds included the chemotherapeutic agents vinblastine, taxol and cyclosporine A and quindine, (an antihypertensive used in 2% of pregnancies, Evesenkov et al., 2006), and saquinavir (an antiretroviral drug used in 0.01-0.3% of pregnancies, Bucala et al., 2000). A 2.47 fold increase in calcein intracellular accumulation was observed, showing the assay is capable of measuring interaction of compounds with P-gp. Evesenko, D., Paxton, J. W. & Keelan, J. A. (2006) Active transport across the human placenta: impact on drug efficacy and toxicity. Expert Opin Drug Metab Toxicol., 2, 51-69.

1599  THE IMPACT OF REPEATED NICOTINE AND ALCOHOL CO-EXPOSURE ON THE IN VIVO CHLORPYRIFOS PHARMACOKINETICS AND PHARMACODYNAMICS.


Chlorpyrifos (CPF) is an organophosphorous insecticide widely used in agriculture. The neurotoxicity of CPF results from inhibition of cholinesterase (ChE) by its metabolite, chlorpyrifos-oxon (CPF-oxon), which subsequently leads to cholinergic hyperstimulation. CPF is known to increase the influence of repeated nicotine and ethanol co-exposure on in vivo CPF pharmacokinetics and pharmacodynamics. The routine consumption of tobacco products and alcoholic beverages may modify key metabolic and physiological processes. Blood and urine profiles of the non-toxic metabolite, 3,5,6-trichloro-2-pyridinol (TCPy) along with changes in plasma and liver ChE activities were measured in male S-D rats (~300 g). Animals were co-exposed to CPF (1 or 5 mg/kg/dy, po), ethanol (1 g/kg/dy, po) and nicotine (1 mg/kg/dy, sc), for 7 days. Rats were sacrificed at times from 1 to 24 hr post-last dosing of CPF. There were apparent differences in blood TCPy pharmacokinetics following nicotine and ethanol pretreatment in both CPF dose groups, which showed higher TCPy peak concentrations and increased blood TCPy AUC (~2-fold) in ethanol/nicotaine pretreated groups compared to saline pretreatment groups. Brain acetylcholinesterase (AChE) activities from ethanol and nicotine-pretreated groups showed substantially less inhibition following repeated 5 mg CPF/kg dosing compared to CPF-only controls (96 ± 13 and 66 ± 7% of naive at 4 hr post-last dosing, respectively). Inhibition of brain AChE activities was minimal in both 1 mg CPF/kg dosing groups, but a similar trend indicating less inhibition following ethanol/nicotine pretreatment was apparent. No alcohol/nicotine treatment effects were observed in plasma ChE activities. This study shows that repeated exposure to alcohol and nicotine (i.e., from smoking) could alter the pharmacokinetics and pharmacodynamics of CPF [Supported by CDC/NIOSH grant R01-OH003629].

1600  THE DEVELOPMENTAL EFFECTS OF LEAD, MANGANESE, AND CHRONIC STRESS ON RAT BEHAVIOR.


Exposure to metals such as lead (Pb) and manganese (Mn) results in neurocognitive deficits in humans and laboratory animals. Children of lower socioeconomic status (SES) are particularly susceptible, as they are more likely to live in homes that contain Pb paint and to be fed soy formula, which contains high levels of Mn. Additionally, these children are also exposed to higher levels of chronic stress, such as neglect, impoverished conditions, or lack of resources. The purpose of this study was to determine the long-term behavioral effects in rats exposed to low-level Pb and/or Mn and chronic stress (i.e., reduced cage bedding) during critical stages of development. It was hypothesized that the concurrent administration of Pb and Mn with the chronic stressor would interact to produce behavioral deficits. On postnatal day (P)4, Sprague-Dawley rat pups were housed in cages containing either no woodchip bedding (paper towel only) or normal woodchip bedding. Pups were gavaged every other day with 10 mg/kg Pb, 100 mg/kg Mn, both Pb and Mn, or an appropriate control from P4-P28, at which time animals were weaned and housed in cages with standard woodchip bedding until the start of behavioral testing on P60. Mn, Pb+Mn, and reduced bedding conditions each resulted in significantly decreased body weights relative to controls, while Pb alone had no effect. Animals raised without bedding also exhibited increased latencies in the Cincinnati water maze, a navigational test of egocentric learning, while metal treatment had little effect on this test. During light-dark exploration, rats raised with reduced bedding demonstrated increased anxiety as they had shorter latencies to dark entry. Animals treated with Mn or Pb+Mn entered the dark more frequently than Pb- or vehicle-treated rats, signifying elevated anxiety. These results suggest that chronic developmental stress and Mn exposure result in behavioral deficits. Ongoing tests will determine whether Pb interacts with Mn and/or developmental stress. Support: RO1ES051689 & T32ES07051

1601  FETAL OXIDATIVE DNA DAMAGE AND REPAIR IN METHYLMERCURY NEURODEVELOPMENTAL DEFICITS.

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Reactive oxygen species (ROS)-mediated oxidative damage to cellular macromolecules has been implicated in embroyopathies caused by several xenobiotics including methylmercury (MeHg), an important environmental toxin that causes neurodevelopmental deficits in infants. We previously found that CD-1 fetuses exposed to a maternal dose of 8 mg/kg MeHg chloride exhibited a dose-dependent increase in oxidative DNA damage by 4 hr post-treatment, measured as 8-oxo-2′-deoxyguanosine (8-oxoG), using high-performance liquid chromatography with electrochemical detection (p<0.05), suggesting that oxidative DNA damage may play a role in the mechanism of MeHg-initiated neurodevelopmental deficits. The studies herein determined the time of peak levels of DNA oxidation in fetal brain and the neurodevelopmental consequences after maternal exposure to MeHg. Pregnant CD-1 dams were injected on gestational day (GD) 17 with either MeHg (4 or 8 mg/kg p.o.) or the phosphate-buffered saline vehicle, sacrificed 2, 6 or 12 hr post-injection, and the fetal brains were collected. Fetal brain levels of 8-oxodG continued to increase at 6 (p<0.05) and 12 hr following the 8 mg/kg MeHg dose, and peaked at 6 hr following the 4 mg/kg dose (p<0.05). In complementary behavioral studies, the offspring of dams treated with 4 mg/kg MeHg on GD 17 were tested for cognitive impairment using an object recognition test at 4 and 6 weeks of age. The 8 mg/kg dose was abandoned due to maternal toxicity. Both genders exposed to MeHg exhibited postnatal cognitive impairment by 6 weeks of age (p<0.05), and males appeared to show cognitive deficits as early as 4 weeks of age. These results show that a dose of MeHg that increases fetal DNA oxidation can also cause postnatal cogni-
tive impairment, suggesting that oxidative DNA damage may play a role in the mechanism of neurodevelopmental deficits caused by in utero exposure to MeHg, with differential gender sensitivity. [Support: NIEHS grant #E05013/48; CHIR; CHIR Rex&D postdoctoral award (GPM)]

1602 EPIGENETIC REPROGRAMMING AND GENE EXPRESSION ALTERATIONS IN RESPONSE TO DEVELOPMENTAL-LEAD EXPOSURE IN MICE.

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The sporadic nature in over ninety percent of Alzheimer’s disease (AD) cases, the differential susceptibility and course of illness, as well as the late age onset of the disease suggests that epigenetic and environmental components play a role in the etiology of late onset AD (LOAD). Published studies from our lab (Basha et al, 2005; Wu et al, 2008; Zawia et al, 2009) showed that lead (Pb) exposure occurring during brain development pre-determined the expression and regulation of AD-related genes later in life, influencing the course of amyloidogenesis and oxidative DNA damage via a process that involved DNA methylation. Thus, interactions between the genome, aging and environmental risk factors impart epigenetic modifications that contribute to AD pathogenesis. These studies focused on single AD-related genes and their regulatory regions; however, a global assessment of gene expression and DNA methylation was not undertaken. An integrated analysis of global gene expression profiles, with simultaneous consideration of both genetic and epigenetic characteristics and of the interactions between these factors and aging, is essential for understanding the complex pathological factors of neurodegeneration. Here we examined across the lifespan genome-wide gene expression and epigenetic profiles in rodents aiming to identify distinguishing epigenetic marks. Initial findings will be discussed.

1603 INCREASED EXPRESSION OF RETINAL GABA TRANSPORTERS AND GABA TRANSMINASE (GABA-T) LIMIT THE ROD-MEDIATED (SCOTOPIC) ELECTRORETINOGRAM (ERG) B-WAVE AMPLITUDE IN MICE WITH GESTATIONAL-LEAD-EXPOSURE (GLE).

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Previously we showed that low-level GLE produced novel and persistent scotopic ERG supernormality in children and adult rats: characterized by an increased amplitude of the a-wave (rod photoreceptor-driven) and b-wave (bipolar cell-driven). Our goals were to analyze scotopic ERGs and determine their cellular/biochemical basis in adult mice with GLE. C57BL/6 female mice were exposed to 55 ppm lead throughout gestation and until postnatal day 10 (PN10), equivalent to human gestation period, and offspring were studied at PN60-70 (adults). Scotopic ERGs were assessed before and one hour after an intravitreal injection of GABA. Gene expression was analyzed by RT-qPCR. Confocal studies used fixed-frozen vertical sections followed by stereological analysis. Peak [BPb] was 20-25 μg/dl at PN10 and was not different from controls by PN30. In GLE mice, unlike rats, the scotopic ERG b-wave was slightly supernormal only at higher stimulus intensities: suggesting GABAergic alterations. In controls, GABA increased the b-wave amplitude at higher stimulus intensities. In GLE mice, GABA had no effect on b-wave amplitudes. GLE selectively increased gene expression of the plasmalemmal and vesicular GABA transporters (GAT3/4 and vGAT, respectively) and the mitochondrial GABA catalyzing enzyme GABA-T. Confocal studies revealed that GABA, relative to controls, increased the: 1) number of rods and bipolar cells with no change in the number of cone photoreceptors, 2) horizontal, amacrine, retinal ganglion or Müller glial cells and 2) expression of GAT3 in Müller glial cells, vGAT in horizontal cell dendrites and GABA-T in mitochondria. In summary, the increased expression of retinal GABA transporters and GABA-T in GLE mice limited the b-wave supernormality, with or without GABA, by reducing the [GABA] at critical outer and inner retinal sites that regulate ERG responses. Supported by NIH Grants ES012482, EY06671, EY07551 and EY07024.

1604 DETRIMENTAL EFFECTS OF LEAD ON HIPPOCAMPAL MECP2 REGULATION AND DNA METHYLATION.


MeCP2 (methyl CpG binding protein 2 gene and its protein product) is a key transcriptional regulator that plays an important role in neuronal maturation, synaptogenesis, dendritic growth and arborization and synaptic plasticity, all processes that are disturbed following developmental lead (Pb) exposure. Alterations in MeCP2 expression/methylation are associated with a variety of cognitive/behavioral disorders including autism, mental retardation, ADHD and learning disabilities. Modifications in MeCP2 may underlie some of the diverse effects of Pb on the nervous system. The phenotypic profile of altered MeCP2 gene function or expression may be dose and time dependent, with different modifications leading to different severity and types of dysfunction. To assess this, post-weaning, male Long Evans rats were maintained on high (1,500 ppm) or low (375 ppm) Pb-containing or control diet for 45 days. MeCP2 mRNA and protein expression were examined in the hippocampus. The high Pb group (blood Pb approx. 30 μg/dl) had a 2 fold decrease in MeCP2 mRNA levels while the low Pb group (approx. 11 μg/dl) had a 1.5 fold decrease in mRNA expression. MeCP2 protein levels were decreased 64% in the high Pb group and 33% in the low Pb group. Methyl-CpG Binding Domain-1 protein (MBD-1) protein levels were decreased almost 50% regardless of amount of Pb exposure. A significant increase (>80%) in global hippocampal DNA methylation was found, regardless of Pb exposure level while activity of DNA methyltransferase 1 (Dnmt1), the enzyme responsible for maintenance of DNA methylation levels was increased almost 2 to 4 fold in low and high Pb groups, respectively. These results show that Pb may cause dose-dependent decreases in MeCP2 expression and increases in Dnmt1 activity while significant alterations in MB1 protein expression and DNA methylation may occur at low Pb levels and may not worsen with larger exposures. These data suggest that low levels of Pb may modify MeCP2 and DNA methylation to an extent that may interfere with normal brain development, maturation, function and plasticity. Supp: ES015295

1605 BEHAVIOR CHANGES AND TRIBUTYL Tin (TBT) CONCENTRATION IN CEREBRUMS OF PREGNANT RATS EXPOSED TO TBT.

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Neurotoxicity is a major toxic effect of tributyltin (TBT). In our previous study, the mean values of F1 rats exposed to TBT via placenta and dam’s milk for total locomotion distance and instance of wall rearing in open field tests were significantly lower than those in the control. Because of the lack of information for dams, the effects of TBT on the dams’ behavior were examined. Pregnant Wistar rats at 9wk of age were exposed to TBT chloride in food at 0 and 125 ppm until 15wk of age. The sporadic nature in over ninety percent of Alzheimer’s disease (AD) cases, the differential susceptibility and course of illness, as well as the late age onset of the disease suggests that epigenetic and environmental components play a role in the etiology of late onset AD (LOAD). Published studies from our lab (Basha et al, 2005; Wu et al, 2008; Zawia et al, 2009) showed that lead (Pb) exposure occurring during brain development pre-determined the expression and regulation of AD-related genes later in life, influencing the course of amyloidogenesis and oxidative DNA damage via a process that involved DNA methylation. Thus, interactions between the genome, aging and environmental risk factors impart epigenetic modifications that contribute to AD pathogenesis. These studies focused on single AD-related genes and their regulatory regions; however, a global assessment of gene expression and DNA methylation was not undertaken. An integrated analysis of global gene expression profiles, with simultaneous consideration of both genetic and epigenetic characteristics and of the interactions between these factors and aging, is essential for understanding the complex pathological factors of neurodegeneration. Here we examined across the lifespan genome-wide gene expression and epigenetic profiles in rodents aiming to identify distinguishing epigenetic marks. Initial findings will be discussed.

1606 PERINATAL ARSENIC EXPOSURE INHIBITS BINDING ABILITY OF GLUCOCORTICOID RECEPTORS TO NUCLEAR RESPONSE ELEMENTS ALTERING GENE EXPRESSION AND AFFECTING LEARNING BEHAVIOR IN C57BL/6 J ADOLESCENT MICE.

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Learning deficits in children following arsenic (As) exposure via drinking water, have been epidemiologically undescribed. Arsenic is a persistent environmental toxin and exposure has been shown to perturb the hypothalamic-pituitary-adrenal (HPA) stress axis. The glucocorticoid receptors (GR) are an integral part of the HPA axis and are found throughout the CNS, particularly in the hippocampus, an area of the brain important in learning and memory. The mitogen-activated protein kinase

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were significantly lower than in those in the controls. At 6wk of age, the mean body weight in TBT-F1 rats was also significantly lower than that in the controls. However, the mean relative thymus weight in TBT-F1 rats was significantly higher, and there were no differences in relative spleen weight between TBT-F1 rats and the controls. The mean value of instance of wall rearing in the open field tests in TBT-F1 rats tended to be lower than in the controls. Cessation of exposure to TBT resulted in quick recovery of weight of the immune system organs, but inhibition of rat development will continue after cessation. TBT-induced neurotoxicity is different according to the rats’ stages of development and their gender.

There is evidence that the effects of lead (Pb²⁺) intoxication on neurodevelopment may be influenced by social factors as well. Children of low socioeconomic backgrounds are more susceptible to the detrimental effects of Pb²⁺ and these same households experience a great deal of stress. Previous studies have shown that developmental Pb²⁺ exposure decreases granule cell neurogenesis in the subgranular zone of the hippocampus dentate gyrus (Verina et al., 2007). These findings provided cellular evidence for the synaptic plasticity and learning deficits in Pb²⁺-exposed animals. In this study, we examined the long-term effects of Pb²⁺ exposure on learning and memory in offspring exposed to Pb²⁺ in utero and nursing. We also investigated the role of neonatal stress in the Pb²⁺-exposed offspring. We used Ki67 as an endogenous marker of proliferation. The results showed that Pb²⁺ exposure during gestation and lactation led to a decrease in Ki67-positive cells in the dentate gyrus of the hippocampus. These findings suggest that exposure to Pb²⁺ can have a lasting impact on offspring.

The developing central nervous system is vulnerable to the toxic effects of lead (Pb²⁺) and is manifested as impaired cognitive function and intellectual capacity. Pb²⁺ acts as a non-competitive NMDA receptor (NMDAR) antagonist, blocking downstream signaling regulations transcription of neurotrophins such as brain-derived neurotrophic factor (BDNF). Recent studies from our lab have shown that exogenous addition of BDNF to hippocampal (Hipp) neurons in culture mitigates Pb²⁺-induced neurotoxicity (Neal and Guirarte, 2009). This suggests that BDNF signaling may be impaired by Pb²⁺ exposure. Using the same primary Hipp neuron culture system, we now show that chronic Pb²⁺ exposure during synaptogenesis significantly decreases Pro-BDNF protein levels relative to controls. BDNF and its pro-forms are mainly expressed by ELISA showed a significant (p<0.05) decrease caused by Pb²⁺ exposure. Immunocytochemistry co-labeling of MAP-2 and Pro-BDNF revealed that Pb²⁺ altered Pro-BDNF expression along dendritic arbors of Hipp neurons. The Huntingtin (Htt) protein regulates vesicular transport including BDNF-containing vesicles. When Htt is phospho in the endoplasmic reticulum (ER), BDNF transport is facilitated, while dephosphorylation favors retrograde transport. Chronic Pb²⁺ exposure significantly decreased pS421Htt compared to controls (p<0.05) in the absence of a change in total Htt protein. A decrease in pS421Htt by Pb²⁺ is likely to alter anterograde transport and release of BDNF. These findings suggest that chronic Pb²⁺ exposure during the period of synaptogenesis of Hipp neurons in culture alters Pro-BDNF protein levels and impairs basal release. A putative mechanism for the Pb²⁺-induced decrease in BDNF release may be mediated by post-translational modification of the Htt protein (Supported by NIEHS grant number ES06189 to TRG).

1609 EFFECTS OF DEVELOPMENTAL LEAD EXPOSURE AND PRENATAL STRESS ON GRANULE CELL NEUROGENESIS IN THE HIPPOCAMPUS DENTATE GYRUS.

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Neurotoxicity is a major effect of tributyltin (TBT). From the results of open field tests, the inhibition of female rats’ activity induced by exposure to TBT via the placenta and dam’s milk and/or food was suggested at 15wk of age in our previous study. In the present study, TBT neurotoxicity in developing male F1 rats at 6wk of age was evaluated by open field tests. Pregnant Wistar rats were exposed to TBT chloride at 0 and 125ppm in food. After weaning, the exposure to TBT was stopped, and the F1 rats were maintained by commercial rodent chow. At 6wk of age, open field tests were performed (n=10/group). The body weight and organ weights (spleen, thymus, liver, and kidney) were determined at weaning and 6wk of age. At weaning, the mean body weight in TBT-F1 rats was significantly lower than that in the controls. The mean relative spleen and thymus weights in TBT-F1 rats were significantly lower than in those in the controls. At 6wk of age, the mean body weight in TBT-F1 rats was also significantly lower than that in the controls. However, the mean relative thymus weight in TBT-F1 rats was significantly higher, and there were no differences in relative spleen weight between TBT-F1 rats and the controls. The mean value of instance of wall rearing in the open field tests in TBT-F1 rats tended to be lower than in the controls. Cessation of exposure to TBT resulted in quick recovery of weight of the immune system organs, but inhibition of rat development will continue after cessation. TBT-induced neurotoxicity is different according to the rats’ stages of development and their gender.

1608 EVALUATION OF TBT NEUROTOXICITY IN DEVELOPING MALE F1 RATS BY OPEN FIELD TESTS.

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1610 ALTERED BDNF PROTEIN EXPRESSION AND RELEASE BY CHRONIC Pb²⁺ EXPOSURE DURING SYNAPTGENESIS IN PRIMARY HIPPOCAMPAL NEURONS.

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The developing central nervous system is vulnerable to the toxic effects of lead (Pb²⁺) and is manifested as impaired cognitive function and intellectual capacity. Pb²⁺ acts as a non-competitive NMDA receptor (NMDAR) antagonist, blocking downstream signaling regulations transcription of neurotrophins such as brain-derived neurotrophic factor (BDNF). Recent studies from our lab have shown that exogenous addition of BDNF to hippocampal (Hipp) neurons in culture mitigates the loss of presynaptic vesicular proteins and reverses the impairment in vesicular release produced by chronic Pb²⁺ exposure (Neal and Guirarte, 2009). This suggests that BDNF signaling may be impaired by Pb²⁺ exposure. Using the same primary Hipp neuron culture system, we now show that chronic Pb²⁺ exposure during synaptogenesis significantly decreases Pro-BDNF protein levels relative to controls. BDNF and its pro-forms are mainly expressed by ELISA showed a significant (p<0.05) decrease caused by Pb²⁺ exposure. Immunocytochemistry co-labeling of MAP-2 and Pro-BDNF revealed that Pb²⁺ altered Pro-BDNF expression along dendritic arbors of Hipp neurons. The Huntingtin (Htt) protein regulates vesicular transport including BDNF-containing vesicles. When Htt is phospho in the endoplasmic reticulum (ER), BDNF transport is facilitated, while dephosphorylation favors retrograde transport. Chronic Pb²⁺ exposure significantly decreased pS421Htt compared to controls (p<0.05) in the absence of a change in total Htt protein. A decrease in pS421Htt by Pb²⁺ is likely to alter anterograde transport and release of BDNF. These findings suggest that chronic Pb²⁺ exposure during the period of synaptogenesis of Hipp neurons in culture alters Pro-BDNF protein levels and impairs basal release. A putative mechanism for the Pb²⁺-induced decrease in BDNF release may be mediated by post-translational modification of the Htt protein (Supported by NIEHS grant number ES06189 to TRG).
The vision for toxicity testing in the 21st century proposes a toxicology based on high throughput screening, in vitro assays, and computational modeling. Using these tools pathways of toxicity shall be identified that provide more accurate predictions of human toxicity than current animal tests. A promising approach for this is metabolomics which studies biochemical processes in biological systems by measuring low molecular weight metabolites. This study aimed to develop an in vitro metabolomics approach for neurotoxicity assessment. Rat primary aggregating brain cell cultures shown to exhibit relevant morphological and functional CNS processes were treated with methyl mercury and caffeine. To study treatment induced metabolic alterations cellular metabolic profiles were acquired by mass spectrometry (MS) and analyzed using principal component analysis (PCA). Results showed concentration dependent cluster formations of methyl mercury samples and treatment dependent formations of caffeine samples at sub-cytotoxic concentrations. Several metabolites responsible for these cluster formations were identified as γ-aminobutyric acid, choline, glutamine, creatine and spermine by MS fragmentation pattern analysis. Quantification of their mass ion intensities showed that methyl mercury and caffeine induced concentration dependent effects related to their mechanisms of action. Next, eight compounds including neurotoxins, hepatotoxins and nephrotoxins were tested at sub-cytotoxic concentrations. PCA analysis showed a cluster formation depending on target organ toxicity indicating the potential of the approach to predict the neurotoxic potency of compounds. Obtained results demonstrate the value of metabolomics to assess in vitro neurotoxicity and identify relevant biomarkers. Ongoing studies address developmental neurotoxicity by measuring metabolic profiles over time to indentify metabolites important for neurodevelopment. In addition, genomics and proteomics technologies are applied to create a systems toxicology approach to identify pathways of toxicity.

Methylmercury (MeHg) has been recognized as a neurotoxicant targeted on the central nervous system including cerebellum and cerebral cortex. Some molecular processes were treated with methyl mercury and caffeine. To study treatment in-duced metabolic alterations cellular metabolic profiles were acquired by mass spectrometry (MS) and analyzed using principal component analysis (PCA). Results showed concentration dependent cluster formations of methyl mercury samples and treatment dependent formations of caffeine samples at sub-cytotoxic concentrations. Several metabolites responsible for these cluster formations were identified as γ-aminobutyric acid, choline, glutamine, creatine and spermine by MS fragmentation pattern analysis. Quantification of their mass ion intensities showed that methyl mercury and caffeine induced concentration dependent effects related to their mechanisms of action. Next, eight compounds including neurotoxins, hepatotoxins and nephrotoxins were tested at sub-cytotoxic concentrations. PCA analysis showed a cluster formation depending on target organ toxicity indicating the potential of the approach to predict the neurotoxic potency of compounds. Obtained results demonstrate the value of metabolomics to assess in vitro neurotoxicity and identify relevant biomarkers. Ongoing studies address developmental neurotoxicity by measuring metabolic profiles over time to indentify metabolites important for neurodevelopment. In addition, genomics and proteomics technologies are applied to create a systems toxicology approach to identify pathways of toxicity.

Elevated Pb exposure and stress are co-occurring risk factors, particularly for low socioeconomic communities. Pb and stress target common biological substrates, including the hypothalamic-pituitary-adrenal axis and brain mesocorticlimbic dopamine and glutamate systems. Both also adversely impact cognition. Our previous studies in rats have shown potentiated effects of combined maternal Pb exposure and prenatal stress (PS) on Fixed Interval performance in female offspring. The current study extended those efforts to examine the impact of exposure to continuous Pb (0 to 50 ppm in drinking water beginning 2 mos prior to dam breeding and continuing across life in offspring, producing blood Pb levels of c3 and 10 μg/dl, respectively) which more closely approximates human exposure conditions, or to prenatal stress, or the combination, on a direct measure of learning, i.e., behavior maintained by a multiple schedule of repeated learning and performance. Food reinforcement on this paradigm requires learning a new sequence of 3 responses each session in the repeated learning component, and completion of the same 3 response sequence each session during the performance component. Pb exposure per se reduced accuracy levels of females in the repeated learning component in a sequence specific manner, i.e., for a sequence of greater difficulty. Moreover, for this sequence, females exposed to Pb + PS, but not to either alone, showed a dramatic increase in the total number of responses (correct + incorrect) required to complete a reinforced sequence, i.e., they learned more slowly, a deficit that gradually disappeared across sessions. This deficit resulted in longer session times for Pb+PS females and therefore a lower reinforcement density as well, which may have sustained the deficits. These findings underscore the critical importance of examining the impact of chemical exposures in conjunction with other extant risk factors with which they share common adverse outcomes for a true assessment of exposure risk. ES012712 and ES012427.

AN IN VITRO METABOLOMICS APPROACH FOR NEUROTOXICITY ASSESSMENT.

The developmental neurotoxicity of silver nanoparticles modeled in PC12 cells.

Elevated Pb exposure and stress are co-occurring risk factors, particularly for low socioeconomic communities. Pb and stress target common biological substrates, including the hypothalamic-pituitary-adrenal axis and brain mesocorticlimbic dopamine and glutamate systems. Both also adversely impact cognition. Our previous studies in rats have shown potentiated effects of combined maternal Pb exposure and prenatal stress (PS) on Fixed Interval performance in female offspring. The current study extended those efforts to examine the impact of exposure to continuous Pb (0 to 50 ppm in drinking water beginning 2 mos prior to dam breeding and continuing across life in offspring, producing blood Pb levels of c3 and 10 μg/dl, respectively) which more closely approximates human exposure conditions, or to prenatal stress, or the combination, on a direct measure of learning, i.e., behavior maintained by a multiple schedule of repeated learning and performance. Food reinforcement on this paradigm requires learning a new sequence of 3 responses each session in the repeated learning component, and completion of the same 3 response sequence each session during the performance component. Pb exposure per se reduced accuracy levels of females in the repeated learning component in a sequence specific manner, i.e., for a sequence of greater difficulty. Moreover, for this sequence, females exposed to Pb + PS, but not to either alone, showed a dramatic increase in the total number of responses (correct + incorrect) required to complete a reinforced sequence, i.e., they learned more slowly, a deficit that gradually disappeared across sessions. This deficit resulted in longer session times for Pb+PS females and therefore a lower reinforcement density as well, which may have sustained the deficits. These findings underscore the critical importance of examining the impact of chemical exposures in conjunction with other extant risk factors with which they share common adverse outcomes for a true assessment of exposure risk. ES012712 and ES012427.

DEVELOPMENTAL NEUROTOXICITY OF SILVER NANOPARTICLES MODELED IN PC12 CELLS.

The growing use of silver nanoparticles (AgNP) in consumer and medical products is raising environmental and human silver exposures. We recently found that Ag+ impairs cell replication and differentiation in PC12 cells, a standard in vitro model for neuronal development. Here, we compared the effects of Ag+ vs. citrate-coated AgNPs. Exposure of undifferentiated cells to either Ag+ or AgNPs for 24 hr produced significant inhibition of DNA synthesis; AgNPs were effective at nominal concentration (total Ag/total volume) as low as 1 μM and showed a maximal effect at 30 μM. With a shorter, 1 hr exposure, AgNPs did not affect DNA synthesis but Ag+ did, indicating that AgNPs require time, either to release Ag+ into the medium or to be taken up by the cells, in order to exert their deleterious effects. A 24 hr exposure to Ag+ also inhibited protein synthesis with a different dose-response relationship from that of DNA synthesis, pointing to additional mechanisms of toxicity. In differentiating cells, a 4 day exposure to Ag+ or AgNPs elicited oxidative stress while decreasing the number of cells, but the dose-response relationships for AgNPs differed for the two effects. Oxidative stress increased monotonically from 10 to 100 μM AgNPs, equal to or greater than the effect of 10 μM Ag+; in contrast, the loss of cells evoked by AgNPs was less than that of Ag+ and did not show a progressive increase with concentration. This suggests that AgNPs have additional effects that can offset the cell loss evoked by oxidative stress, perhaps involving suppression of apoptosis as previously found for low concentrations of Ag+. Our results provide some of the first evidence that AgNPs impair development in undifferentiated and differentiating neuroontic cells. Our future work will compare these effects with AgNPs of different size and coating in both PC12 cells and zebrafish.

Mitochondrial dysfunction in an APP/PS1 mouse model of Alzheimer’s disease.

An intensively investigated hypothesis of human neurodegenerative disease involves the role of mitochondrial dysfunction. The mitochondrial electron transport chain (ETC) complexes have been determined to be sensitive targets in these debilitating diseases: Complex 1 dysfunction with Parkinson’s disease, Complex II with Huntington’s disease and Complex IV with Alzheimer’s disease (AD). In AD, over-expression of human mutant forms of amyloid precursor protein (APP) and presenilin 1 (PS1) in mouse models have been used to examine indices leading to disease pathology. In this study, we assessed oxidative phosphorylation in isolated non-synaptic mitochondria from APP/PS1 mouse brain and their wildtype litter mates. We found that MeHg downregulated the expression of Rac1 and Cdc42 but did not affect RhoA. The exposure concentration and time course studies confirmed that Rac1 is targeted during an early stage of MeHg-induced cytotoxicity. The results indicate that neuritic degeneration, in particular axonal degeneration triggered by the downregulation of Rac1 expression, contributes to MeHg-induced apoptotic cell death in cultured cerebrocortical neurons.

Potentiated impairment of learning in female offspring exposed to continuous lead (Pb) and prenatal stress.

Elevated Pb exposure and stress are co-occurring risk factors, particularly for low socioeconomic communities. Pb and stress target common biological substrates, including the hypothalamic-pituitary-adrenal axis and brain mesocorticlimbic dopamine and glutamate systems. Both also adversely impact cognition. Our previous studies in rats have shown potentiated effects of combined maternal Pb exposure and prenatal stress (PS) on Fixed Interval performance in female offspring. The current study extended those efforts to examine the impact of exposure to continuous Pb (0 to 50 ppm in drinking water beginning 2 mos prior to dam breeding and continuing across life in offspring, producing blood Pb levels of c3 and 10 μg/dl, respectively) which more closely approximates human exposure conditions, or to prenatal stress, or the combination, on a direct measure of learning, i.e., behavior maintained by a multiple schedule of repeated learning and performance. Food reinforcement on this paradigm requires learning a new sequence of 3 responses each session in the repeated learning component, and completion of the same 3 response sequence each session during the performance component. Pb exposure per se reduced accuracy levels of females in the repeated learning component in a sequence specific manner, i.e., for a sequence of greater difficulty. Moreover, for this sequence, females exposed to Pb + PS, but not to either alone, showed a dramatic increase in the total number of responses (correct + incorrect) required to complete a reinforced sequence, i.e., they learned more slowly, a deficit that gradually disappeared across sessions. This deficit resulted in longer session times for Pb+PS females and therefore a lower reinforcement density as well, which may have sustained the deficits. These findings underscore the critical importance of examining the impact of chemical exposures in conjunction with other extant risk factors with which they share common adverse outcomes for a true assessment of exposure risk. ES012712 and ES012427.
leading to an overall decrease in respiratory control ratios. Protein levels for Complex IV decreased with age in female APP/PS1 mice. Conversely, APP levels in both male and female APP/PS1 animals were increased as compared to wildtypes. In the organotypic hippocampal slices, basal and maximal oxygen consumption, as well as rates following inhibition with antimycin A could be stably measured over several hours suggesting this methodology could be useful for mitochondrial respiration measurements in tissue, not presently an option using the Clark electrode. Future respiration studies with the extracellular flux analyzer will be applied using the hippocampal slice model in AD transgenic mice.

1616 EFFECT OF AGING ON EXPRESSION OF HEPATIC TRANSPORTERS AND BILE ACID SYNTHESIZING ENZYMES IN MOUSE LIVER.

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Aging is a process accompanied by increasing macromolecular damage and declining physiological functions in various organs. Most of the studies on age-related gene expression have focused on drug-metabolizing enzymes and only limited to a couple of age groups. Very little information is available on the effect of aging on hepatic transporters. Using multiplex suspension assays, we determined the expression profiles of transporters and bile acid synthesizing enzymes in livers of male and female C57BL/6 mice of 10 age groups, ranging from 3 to 29 months of age. The uptake transporter Oatp1a1 showed the most dramatic decrease with age, starting at 3 months of age. A more gradual decrease with age was observed in other hepatobiliary uptake transporters, starting at about 9 months of age. Most of the efflux transporters had a decrease in expression after one year, such as Mrp2, Mrp6, Bsep, Abcg5 and Abcg8, whereas Mrp4 increased dramatically in female mice with aging. Bialk, Bsep, Cyp7b1, Cyp8b1, and Cyp27a1 decreased gradually with age; Cyp7a1 decreased only after 15 months, whereas Cyp39a1 increased with age. The majority of the genes studied showed significant gender differences in expression throughout the aging process, including male-predominant Oatp1a1, Bsep, Cyp7b1 and Cyp8b1, as well as female-predominant Oatp1a4, Oatp2b1, Oct1, Enr1, Ntcp, Mate1, Mdr2, Mrp3, Mrp4, and Cyp39a1. Taken together, these data suggest a potential explanation for altered pharmacokinetics and pharmacodynamics of therapeutic drugs in the elderly, as well as increased cholesterol levels due to decreased conversion of cholesterol to bile acids. Understanding the effects of aging on drug processing genes may provide useful information in determining the effective and safe dosages of drugs for the elderly. (Supported by NIH grants ES009649, ES013714, ES009716, ES007079, and RR021940)

1617 HEPATOCELLULAR SPECIFIC DELETION OF NADPH-CYTOCHROME P450 REDUCTASE (H-CPR-NULL) IN MICE DISTURBS BILE-ACID HOMEOSTASIS BY MINIMIZING THE CLASSICAL PATHWAY OF BILE ACID BIOSYNTHESIS.

X. Cheng, Y. Zhang and C. D. Klaassen. Pharmacology, Toxicology, and Therapeutics, KUMC, Kansas City, KS.

Cyp7a1 and 8b1, two critical enzymes for the classic microsomal route of bile-acid (BA) biosynthesis, requires Cpr for their activities. The alternative BA biosynthesis pathway is initiated by mitochondrial Cyp27a1, and the rate-limiting step in this pathway is catalyzed with taurine. The bile acid profiles in mouse liver were similar between CA, DCA, and CDCA feeding, whereas CDCA feeding mainly suppressed the classic pathway. Gender differences in expression throughout the aging process, including male-predominant Oatp1a1, Bsep, Cyp7b1 and Cyp8b1, as well as female-predominant Oatp1a4, Oatp2b1, Oct1, Enr1, Ntcp, Mate1, Mdr2, Mrp3, Mrp4, and Cyp39a1. Taken together, these data suggest a potential explanation for altered pharmacokinetics and pharmacodynamics of therapeutic drugs in the elderly, as well as increased cholesterol levels due to decreased conversion of cholesterol to bile acids. Understanding the effects of aging on drug processing genes may provide useful information in determining the effective and safe dosages of drugs for the elderly. (Supported by NIH grants ES009649, ES013714, ES009716, ES007079, and RR021940)

1618 LIVER BILE ACID METABOLISM IN MALE AND FEMALE C57BL/6 MICE FED BILE ACID-SUPPLEMENTED DIETS.

Y. Zhang and C. D. Klaassen. Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

An improved UPLC-MS/MS method was established for the simultaneous analysis of bile acids and kero bile acids, as well as their glycine, taurine, sulfate, and glucuronon conjugates in mouse livers. This simple and sensitive method was validated and applied to investigate liver bile acid metabolism in both male and female C57BL/6 mice fed various diets supplemented with 1% cholic acid (CA), 0.3% deoxycholic acid (DCA), 0.3% chenodeoxycholic acid (CDCA), 0.3% lithocholic acid (LCA), 3% ursodeoxycholic acid (UDCA), or 2% cholesteramine (resin). Results from these studies indicate that bile acids were biotransformed by the intestinal bacteria before they entered the liver, where the majority of them were conjugated with taurine. The bile acid profiles in mouse liver were similar between CA and DCA feeding, as well as between CDCA and LCA feeding. CA feeding suppressed both the classic and alternative pathways of liver bile acid biosynthesis, whereas CDCA feeding mainly suppressed the classic pathway. Gender differences of liver bile acid composition were observed after CA, DCA, CDCA, and LCA feedings, but were not prominent after UDCA feeding. Sulfation of CA or CDCA was at the 7-OH position, and was increased by CA or CDCA feeding in male mouse livers more than in females. In contrast, sulfation of 12-oxo bile acid (TLCA) at the 3-OH position was female predominant, and increased by UDCA and LCA feedings. In addition, feeding the resin also increased sulfaation of CA and CDCA. Glucuronidation of bile acids was a minor bile acid metabolic pathway in mice, which was detected only after UDCA feeding. In summary, the present systematic study on liver bile acid metabolism will aid in interpreting bile acid-mediated gene regulation, as well as hepatotoxicity and therapeutic use of various bile acids. (Supported by NIH ES009716, ES013714, ES009649, RR021940)

1619 BILE ACIDS INCREASE PROINFLAMMATORY GENE EXPRESSION IN HEPATOCYTES BY EARLY GROWTH RESPONSE FACTOR-1-DEPENDENT AND INDEPENDENT MECHANISMS.

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Cholestasis occurs when excretion of bile acids from the liver is interrupted causing liver injury in both humans and animals. Recent studies have shown that inflammation exacerbates injury during cholestasis. However, the molecular mechanism by which cholestasis drives inflammation in the liver is not known. Data from our lab has shown that early growth response factor-1 (EGR-1) regulates cholestasis via upregulation of proinflammatory mediators that mediate liver injury in both humans and animals. Recent studies have shown that inflammation causes cholestasis. In human and animal studies, proinflammatory mediators induced by cholestasis cause liver injury. However, the mechanisms by which proinflammatory mediators increase in hepatocytes during cholestasis are not well understood. We have previously shown that proinflammatory mediators such as TNF-α, IL-6, IL-10, and others increase expression of proinflammatory mediators in hepatocytes in response to bile acid exposure, and that these effects are mediated by EGR-1. In this study, we investigated the role of EGR-1 in regulating proinflammatory genes in human hepatocytes exposed to bile acids. We found that proinflammatory genes such as TNF-α, IL-6, IL-10, and others increased in hepatocytes exposed to bile acids, and that these effects were mediated by EGR-1. In conclusion, our results provide new insights into the molecular mechanisms by which proinflammatory mediators increase in hepatocytes during cholestasis, and suggest a potential therapeutic target for the prevention of liver injury in this setting.

1620 THROMBIN SIGNALING ENHANCES TGF-BETA INDUCTION OF INTEGRIN BETA6 IN BILE DUCT EPITHELIAL CELLS.

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Damage to intrahepatic bile ducts causes cholestasis, inflammation, coagulation cascade activation and liver fibrosis. Recent studies have shown that the αvβ6 integrin, which activates the fibrogenic cytokine TGF-β, contributes to liver fibrosis.
Expression of the β6 integrin (Itgβ6) is restricted to bile duct epithelial cells (BDECs) in the liver after common bile duct ligation. However, the mechanisms of Itgβ6 mRNA regulation in BDECs have not been identified. We tested the hypothesis that coagulation protease signaling induces Itgβ6 mRNA expression in BDECs. Treatment of transformed human BDECs (MMNK-1) with thrombin or protease activated receptor-1 (PAR-1) agonist peptides (TFLLRN or SFLLRN) induced the expression of interleukin 8 (IL-8), a chemokine induced by PAR-1 sig- naling. However, neither thrombin nor agonist peptides increased Itgβ6 mRNA levels in MMNK-1 cells. In contrast, treatment of the MMNK-1 cells with TGF-β increased Itgβ6 mRNA levels without affecting IL-8 expression. As thrombin and TGF-β are likely generated concurrently during cholestatic liver injury, we deter- mined whether thrombin enhanced TGF-β-induced Itgβ6 mRNA expression. Co-treat- ment with either thrombin or the agonist peptides significantly enhanced TGF-β induction of Itgβ6 mRNA, whereas co-treatment with a scrambled peptide (FS- LLRN) was without effect. Thrombin, but not TGF-β, activated the p38 and JNK1/2 MAPK signaling pathways. Itgβ6 mRNA induction in cells co-treated with thrombin and TGF-β was significantly reduced by a p38 inhibitor (SB203580) and a JNK1/2 inhibitor (SP600125). Taken together, the results sug- gest that thrombin activation of p38 and JNK1/2 signaling enhances TGF-β de- pendent induction of the Itgβ6. Further elucidation of the mechanisms whereby these fibrogenic mediators synergistically induce the Itgβ6 and contribute to fibro- genesis may reveal novel strategies for the treatment of cholestatic liver disease.

### 1621 ONTOGENY OF HEPATIC EFFLUX TRANSPORTERS IN OBESE MICE.

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ATP binding cassette (ABC) transporters are a family of efflux transporters in liver important for the excretion of xenobiotics. Ob/ob mice are a congenic mouse strain that have a spontaneous mutation in gene that encodes leptin. Phenotypical changes in ob/ob mice are observed at week 4, with marked ABC transporter expression changes in liver by week 11, as compared to lean control mouse (C57BL/6). The ontogeny of ABC transporter expression in ob/ob mice has not been described, but may point to factors responsible for inducing transporter expression in obesity. The purpose of this study is to correlate changes in liver ABC transporter expression with phenotype changes and liver pathology in ob/ob and lean mice. Ob heterozy- gous mice were bred and their progeny were genotyped to confirm leptin mutation and gender. Liver from male and female lean and ob/ob homozygous mice litter- mates at 1, 3, 4 and 8 weeks of age were collected and mRNA was quantified. Expression of Abcc3 and 4 in males and females ob/ob mice increased with age. Abcc3 expression in both males and females decreased with age, whereas Abcc6 and Abcb1a expression decreased with age in only males ob/ob mice. At week 1, Abcc3 and 4 mRNA expression was downregulated in ob/ob compared to lean, whereas this observation was reversed at 3, 4 and 8 weeks of age. Abcg2 expression in ob/ob male was same as in lean at week 1, but its expression was upregulated in both male and female mice at 3, 4 and 8 weeks as compared to lean mice. Abcg2 expres- sion in lean mice remained fairly constant in all age groups but increased in ob/ob mice with age. In conclusion, ontogeny of hepatic efflux transporter expression in ob/ob mouse is different from lean mice, with week 3 and 4 being the time points where changes in the expression pattern were observed. Thus far, our data indicates that week 3 and 4 are pivotal time points for the upregulation of ABC transporters during obe- sity and indicate that leptin signaling along with physiological changes associated with the onset of obesity are important factors for regulation of transporter expres- sion in liver.

### 1622 LONG-TERM EFFECT OF A HIGH CHOLESTEROL DIET ON HEPATIC DRUG TRANSPORTER EXPRESSION IN AKR AND C57BL/6 MICE.

M. A. Paranjo and A. Slitt. Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI.

Obesity is a major concern in the United States, with its major cause being con- sumption of a diet rich in fat and cholesterol (CH). High CH levels are known to cause liver and gallbladder disease. Combined obesity and CH may further lead to gallbladder cancer and cholestasis. The purpose of this study was to determine the long-term effect of feeding lithogenic diet on drug transporter expression in 2 strains of mice which differ in their susceptibility towards CH crys- tal formation in gallbladder bile. Studies have shown that AKR mice are resistant, whereas C57BL/6 mice are susceptible to CH gallstone formation. Adult male AKR and C57BL/6 mice were fed with lithogenic (15% fat, 1.25% CH, 0.5% sodium cholate) or standard diet (5% fat) for 8 wks, then gallbladders, livers and blood were collected. The lithogenic diet resulted in increased crystal formation in the gallbladder bile of C57BL/6 mice as compared to AKR. Serum CH levels increased with the lithogenic diet in both the strains of mice but no strain difference was ob- served. The total RNA was isolated from liver and the mRNA expression of trans- porters like multidrug resistance-associated proteins (Mrps) and organic anion transporting polypeptides (Oatps) was determined using Quantigene 2.0 plus assay. The lithogenic diet increased Oatp1a4, and 1b2 mRNA levels in livers of AKR mice and decreased Oatp1a4 levels in livers of C57BL/6 mice. The lithogenic diet increased Mrp1 in both the strains. Mrp2, 3 and 4 mRNA expression was upregu- lated in livers of the AKR mice, but Mrp 3 and 4 were downregulated in livers of the C57BL/6 mice. Nuclear factor E2 related factor 2 (Nrf2) is a transcription fac- tor that regulates the expression of many enzymes and transporters in liver that me- diate metabolism and transport processes. The lithogenic diet significantly induced the Nrf2 mRNA expression in AKR mice but not in the C57BL/6 mice, which could suggest the possibility of Nrf2 playing a role towards the resistance of gall- stone susceptibility. (NIH K0813782A, 5R01ES016042-02)

### 1623 CHANGES IN EXPRESSION OF LIVER MRP2 EXPRESSION IN MOUSE MODELS OF FASTING AND CALORIC RESTRICTION.

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Epidemiological and clinical studies show that obesity changes disposition of vari- ous drugs. Mouse models of obesity demonstrate altered drug metabolizing enzyme and drug transporter expression. Multidrug resistance-associated protein 2 (Mrp2) is a hepatic transporter important in transport of conjugates of various drugs and endogenous as well as environmental compounds. Altered expression of Mrp2 changes disposition of acacetaminophen, morphine and thyroid hormone conjugates in rodent models of disposition. CDC estimates that two thirds of American population is undergoing caloric restriction (CR) / fasting to combat obesity. Thus, it is important to study dispositional and transcriptional cir- cuitry changes in both obese and lean individuals undergoing nutrient deprivation. The purpose of this study was to understand changes in (1) expression of Mrp2 and (2) binding of transcription factors (TF) important in xenobiotic metabolism as a function of fasting or CR in C57BL/6 (lean) and ob/ob (obese/OB) mouse models. Lean and ob/ob mice were fasted for 30 hours or put on a 40% reduced caloric diet for a period of 10 weeks. Fasting did not affect mRNA or protein expression of Mrp2 in livers of either mouse model. CR increased Mrp2 mRNA expression by about 1.2 and 2 folds in lean and OB CR mice over fed controls, respectively. Changes in TFs binding to their consensus response elements (RE) were analyzed using liver nuclear extracts from the CR mice. Obesity increased Ahr, Nrf2, Nrfl2, CREB, and PPAR binding by 147-275 percent to their respective RE as com- pared to lean fed controls. This increased binding in hepatic nuclear fractions from OB mice was reversed by CR. In summary, our data indicate that CR can restore Mrp2 expression in liver to that detected in livers of lean mice, and this is associated with changes in several TFs. Overall, these data indicate that there is potential to re- store hepatic disposition changes associated with obesity through caloric restriction. (KES013782A, RES016042A)

### 1624 TRANSPORTER EXPRESSION IN DB/DB AND DIET- INDUCED OBESE (DIO) MICE.

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Diabetics are found to have altered pharmacokinetic and toxicological profiles for certain drugs. As transporters play important role in drug disposition, it is impor- tant to characterize transporter expression in diabetes. In the present study, trans- porter expression (mRNA and protein) in livers and kidneys of adult male and fe- male db/db mice, as well as male diet-induced obese (DIO) mice was determined. Db/db mice, which have a spontaneous mutation in the leptin receptor, show in- creased body weight, elevated hyperglycemia, and hyperinsulinemia. DIO mice are fed high fat diet (60% fat) for about 12 weeks of age; they have slightly increased body weight, mild hyperglycemia and hyperinsulinemia. Expression of uptake trans- porters was decreased in livers and kidneys of db/db mice, including organic anion transporting polypeptide (Oatp) 1a1, 1a4 and organic anion transporter 2. Oatp1a1 mRNA expression was almost non-detectable in livers and kidneys of db/db male mice compared to wild type. In general, expression of multidrug resist- ance-associated protein (Mrp) efflux transporters was significantly increased in livers of db/db mice. Specifically, Mrp3 and 4 mRNA and protein levels increased greater than 2 fold in db/db male mice livers as compared to wild type mice. Mrp3 mRNA expression was markedly down regulated in kidneys of male db/db mice as compared to wild type. Similarly, in the livers of DIO mice, Mrp3 and 4 mRNA expressions were increased 3.3 and 1.4 fold as compared to that in controls. In DIO mice, uptake transporter expression in liver and kidney, as well as kidney Mrp expression remained unchanged. In summary, expression of numerous transporters
was significantly altered in livers and kidneys of db/db mice. Because some transporters in mouse and human are regulated similarly, and also share some substrate drugs, it should be investigated as to whether diabetic humans might also have altered transporter expression. Db/db mice are more severe model of diabetes as compared to DIO mice, and this likely contributes to the observed changes in transporter expression.


Non-alcoholic fatty liver disease (NAFLD) is initiated by a series of pathological changes in steatosis, inflammation, and fibrosis which can progress to non-alcoholic steatohepatitis (NASH). This progression within NAFLD is considered a "two-hit" model of pathogenesis where the 'first hit' is characterized simply by the deposition of fat in the liver (steatosis). The 'second hit' transitioning to NASH involves the production of reactive oxygen species (ROS) or cytokine release. This study was designed to determine the hepatic global gene expression changes due to the progression of human NAFLD. We examined the gene expression differences between four clinically defined pathological groups: normal, steatotic (>10% lipid deposition), NASH fatty (>5% lipid deposition, inflammation and fibrosis), and NASH not fatty (<5% lipid deposition, inflammation and fibrosis). Individual human liver tissue samples were processed by Affymetrix GeneChip® 1.0ST arrays providing adequate coverage of 33,252 genes represented by 26 probes across the length of each gene. Expression level differences were evaluated at the 0.01 false discovery rate threshold. Analysis revealed 1,319 gene alterations between steatotic and NASH groups while only 22 changes occurred between normal and steatotic groups. Additionally, a principal component analysis utilizing all genes indicated the separation of the samples into two distinctive groups (normal and steatosis; NASH fatty and NASH not fatty). These results suggest that the majority of changes in gene expression occur with the "second hit" in the transition from steatosis to NASH. This finding may help in the identification of an appropriate biomarker for the diagnosis of NASH.

1626 ALTERED EXPRESSION AND REGULATION OF EFFLUX DRUG TRANSPORTERS IN THE PROGRESSION OF HUMAN NON-ALCOHOLIC FATTY LIVER DISEASE. R. N. Hardwick, C. D. Fisher, M. J. Canet and N. J. Cherrington. Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

The expression and regulation of hepatic transport proteins can have a profound effect on the uptake and efflux of drugs in the overall process of drug management. ATP-binding cassette transporters located in the sinusoidal and canalicular membranes of hepatocytes regulate the efflux of drugs and metabolites into blood and bile, respectively. Changes in the expression or functionality of these transporters during liver disease may lead to a greater risk of adverse drug reactions, toxicity, or pharmacologic failure. Non-alcoholic fatty liver disease (NAFLD) is a progressive condition ranging from the relatively benign steatosis to the more severe non-alcoholic steatohepatitis (NASH). Here, we present an analysis of the effect of human NAFLD disease progression on the major efflux drug transport proteins ABCB1, ABCB1, ABCB1 and ABCB2. Human liver samples diagnosed as normal, steatotic (>10% hepatocyte fat deposition), NASH fatty (inflammation, fibrosis and >5% fat deposition), and NASH not fatty (inflammation, fibrosis and <5% fat deposition) were obtained from the NIH Liver Tissue Procurement and Distribution System. The mRNA expression of ABCB1, ABCB4, ABCB5 and ABCB2 was increased in both stages of NASH, but not steatosis. Similarly, protein levels of ABCB1, ABCB2 and ABCB1 were increased in both stages of NASH. There was a significant rise in ABCB4 protein levels only in NASH (fatty); and ABCB5 protein levels were increased in all stages of NAFLD. Immunohistochemical staining also revealed an alternative mechanism of ABCB2 regulation in NASH whereby the transporter protein appears to be internalized away from the canalicular membrane in NASH (not fatty) samples. The increased expression of multiple efflux transporter systems, as well as the altered cellular localization of ABCB2 in NASH, may have profound effects on the ability of patients with NASH to eliminate drugs in an appropriate manner and could contribute to an increased risk of adverse drug reactions.

1627 THE EFFECT OF DIETARY FAT CONTENT ON TCDD-ELICITED HEPATOTOXICITY IN C57BL/6 MICE. B. D. Mets1, A. K. Kopce1, J. R. Harkema1, and T. Zacharewski1,2

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an environmental toxicant that elicits a wide range of pathologies. To investigate the effects of dietary fat on the previously characterized TCDD-elicited fatty liver phenotype, immature, ovariec-tomized (ovx) C57BL/6 mice were provided free access to standard mouse chow diet as well as isoalcoholic diets with varied lipid and carbohydrate content such that 5%, 10%, and 15% of total energy came from fat, while protein content was fixed. Access to the diets was provided three days prior to treatment and throughout the duration of the study. Animals were gavaged once with 30 μg/kg TCDD or sesame oil vehicle, and sacrificed 24 or 168 h post treatment. No significant differences in body weight gain were seen. However, relative liver weight (RLW) was significantly increased in all TCDD-treated groups compared to respective vehicles, except for the 10% fat isoalcoholic diet at 24 h. Interestingly, the 5% fat diet induced the largest increases in RLW. Oil red O and H&E staining showed marked increases in lipid accumulation and vacuolization, with the 5% fat diet eliciting the most dramatic accumulation of fat. Gas chromatography/mass spectrometry also confirmed that there were significant increases in total fatty acid content in all TCDD-treated groups with the 5% diet exhibiting the greatest fat accumulation. In contrast, dietary essential fatty acids, 18:2n6 and 18:3n3 showed an opposite trend, with greater accumulation in the 15% fat diet groups than the respective 5% fat diet groups at 168 h, suggesting that increased dietary fat does lead to modulation of hepatic fatty acid composition, but these changes are minor compared to the overall toxic response. Taken together, these data suggest dietary caloric composition has an effect on the hepatotoxicity of TCDD. Funded by SBRP P42ES04911.

1628 HEPATIC STEATOSIS AND HYPERTRIGLYCERIDEMIA IN TCDD TREATED WILD TYPE AND SCD1(-/-) C57BL/6 MICE. M. M. Angrish1, B. D. Mets2, and T. R. Zacharewski1,2,3

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Previous studies have shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) elicits hypertriglyceridemia, increased serum free fatty acids, and hepatic lipid accumulation due to alterations in lipid transport and metabolism. Stearoyl-CoA desaturase -1 (Scd1), the primary hepatic desaturase, is the rate-limiting step in the synthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acids (FFAs). To further investigate the role of Scd1 in fatty liver hepatotoxicity, comparative studies were conducted in 4-week-old female Scd1 wild type (Scd1+/+), heterozygous (Scd1+/−) and homozygous null (Scd1−/−) mice treated with 30 μg/kg TCDD for 24 and 72 h. Relative liver weights increased across all genotypes and time points with Scd1−/− mice exhibiting the greatest increases while Scd1+/+ mice exhibited the most severe hepatic vacuolization. Hepatic triglyceride analysis indicated that TCDD induced lipid accumulation in a gene-dose dependent manner at both time points (17.61 and 19.11, 14.50 and 18.85 and 11.76 and 12.75 mg/dl triglyceride per gram tissue in Scd1+/+, Scd1+/− and Scd1−/− at 24 h and 72 h, respectively. Gas chromatography/mass spectrometry analysis of hepatic extracts from wild-type mice revealed significant increases in MUFAs that was not observed in null mice. Furthermore, TCDD exposure induced Scd1 protein levels approximately 3-fold and activity 2.9-fold in wild-type mice compared to vehicle controls. Collectively, these results suggest that Scd1 serves a protective role in TCDD elicited hepatic steatosis and hypertriglyceridemia. This work was funded by SBRP P42ES04911.

1629 SYNERGISM BETWEEN ‘OMICS’ WHAT TRANSCRIPTOMICS AND METABOLOMICS REVEAL ABOUT TCDD EFFECTS IN RAT AND MOUSE LIVER.

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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) elicits a broad range of species-specific effects. We previously reported TCDD effects on genomic and hepatic metabolites in immature ovariectomized C57BL/6 mice (30 μg/kg TCDD) and SD
rats (10 mg/kg TCDD). Here we report the relationship between transcriptionists and metabolomics in evaluating species-specific effects. Metabolomics included 13C, 1H and 31P NMR of liver extracts. Transcriptionists used cDNA microarrays and qRT-PCR. Principal Components Analysis (PCA) of computationally-processed multivariate NMR data showed separation for controls vs. TCDD-treated animals in both species, with different NMR peaks accounting for the separation in each species. Primary differences include the development of fatty liver in mice and disruption in hepatic choline metabolism in rats. Mice metabolomics showed a 45% increase in n6 fatty acids (FA) and 3-fold elevation in triglyceride (TAG). Concomitant genomica revealed decreased mRNA expression for FA synthesis and mitochondrial glycerol-3-phosphate dehydrogenase (G3PD), and increase in phospholipase A2. This suggests that TCDD causes increased liver free FA s in mice, leading to decreased FA synthesis and increased TAG synthesis. We hypothesize that the effect on G3PD expression may be related to preferential use of G3P in TAG synthesis. In rats, TCDD induced a 2-fold increase in hepatic sphingosine kinase and choline kinase mRNA expression. Concomitant metabolic changes included a 53% decrease in sphingomyelin, 3-fold elevation in phosphocholehol, and decreased betaine. This suggests altered choline metabolism. Coupled metabolomics and transcriptomics provide links to TCDD-altered pathways, and promises to reveal mechanisms and relationships of gene expression to metabolite profiles. Funding: NIH-R01-E0103927.

1630 COMPARATIVE TOXICOGENOMIC EXAMINATION OF PPARα-REGULATED HEPATIC RESPONSES IN VIVO AND IN VITRO.

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The peroxisome proliferator-activated receptor α (PPARα) is a ligand activated transcription factor that regulates a variety of biological processes including lipid metabolism and energy homeostasis. Peroxisome proliferators (PPs) are non-genotoxic carcinogens in rodents, but not in humans. In this study, we examined gene expression responses elicited by PPs in C57BL/6 mice liver compared to responses in human liver stem-like HL-1 and HepG2 cells. Mice were gavaged once with sesame oil, 300mg/kg clofibrate (CLO) or Wy-14,643 (WY) for 2,4,8,12,18 or 24h, or every 24h and sacrificed after 1,4 or 14days. No significant changes in body weight gain were observed, however, increased relative liver weights were observed after 4 and 14days after WY exposure. Hepatic gene expression monitored using Agilent whole-genome microarrays identified 719 and 1,443 unique genes in response to CLO and WY, respectively (fold change>1.5, P<0.05). Functional analysis identified differentially expressed genes were associated with lipid metabolism, transport, cell cycle and immune response. Using relaxed statistical criteria, 90% of CLO and 75% of the WY differentially expressed genes were in common.

1631 SELECTIVE UPR SIGNALING IN A RODENT MODEL OF CHRONIC ETHANOL INGESTION.

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Chronic ethanol consumption remains the predominant cause of liver injury in the United States. Currently, the precise mechanisms behind the progression of alcoholic liver disease (ALD) are poorly understood, however, numerous processes have been implicated, including enhanced lipid peroxidation, increased generation of reactive oxygen species (ROS), induction of the cytochrome P450s, and stress of the endoplasmic reticulum (ER). In the past decade, ER stress has been implicated in numerous disease states, most notably ALD. ER stress is broadly characterized as a build-up of unfolded or misfolded proteins within the ER. This stress response, in part, is associated with dysregulation of Ca2+ flux changes in post-translational modifications (i.e. glycosylation), altered enzymatic activity and/or errors in disulfide bond generation. Perturbations in ER protein folding results in numerous signaling cascades, termed the Unfolded Protein Response (UPR). These pathways are designed to decrease global protein translation while upregulating key enzymes responsible for antioxidant defenses, protein folding and lipid metabolism. Although studies in rodents have implicated UPR signaling following chronic ethanol consumption, the precise pathways and mechanisms have yet to be elucidated. Utilizing a rodent model for ALD, UPR signaling was assessed with standard immunoblotting and immunohistochemistry in the livers of rats chronically fed ethanol. Among proteins altered, MTP SUBB2-2 and Insig-1 provide a likely mechanism for the observed increase in lipid accumulation. Enhanced generation of the lipid peroxidation product 4-hydroxynonanen (4-HNE) was also observed in this model, and covalent modification of ER-resident proteins by this aldehyde likely plays a role in the accumulation of unfolded protein. In summary, covalent modification of ER-resident proteins by 4-HNE provides compelling evidence for the induction of the ER stress response, contributing to the pathological consequences associated with chronic ethanol ingestion. (Supported by R37 NIH AA009300 & F31 AA018666).

1632 CHRONIC ALCOHOL AND CIGARETTE SMOKE INCREASE HEPATOTOXICITY IN HYPERLIPIDEMIC APOE KNOCKOUT MICE.

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Epidemiologic and clinical studies suggest hyperlipidemia and exposure to cigarette smoke have additive or synergistic effects to increase the risk and severity of alcoholic liver disease; however, the molecular mechanisms responsible for enhanced hepatotoxicity are unknown. We hypothesize that combined exposure to alcohol and environmental tobacco smoke (ETS) on a hyperlipidemic background increases liver toxicity through nitrative stress, hypoxia, and mitochondrial dysfunction. To address this question, male apo knockout mice were exposed to a diet containing ethanol (5% w/v), ETS alone (10 mg total suspended particulate per cubic meter), or a combination for 6 wk. Histologic and functional endpoints were compared to filtered air, ethanol-naive controls. While ethanol consumption induced mild steatosis, combined exposure to ethanol + ETS resulted in enhanced steatosis, inflammation, alpha smooth muscle actin, and collagen levels. Combined exposure to ethanol + ETS induced the largest increase on CYP2E1 and INOS protein, as well as amplified 3-nitrotyrosine and mtDNA damage, and decreased cytochrome c oxidase protein compared to all other groups. Redox active protein thiol content was also decreased in liver mitochondria from ethanol + ETS. Likewise, increased HIF-1 alpha expression was observed in the ethanol + ETS group indicating enhanced hypoxia. These studies demonstrate that ETS increases ethanol-dependent steatosis, hypoxia, nitrative stress, and mitochondrial damage. Therefore, ETS on a background of preexisting hyperlipidemia has the potential to accelerate and amplify alcoholic liver disease.

1633 CHRONIC ALCOHOL INCREASES SENSITIVITY TO MITOCONDRIAL CALCIUM OVERLOAD LEADING TO ENHANCED SUSCEPTIBILITY TO PERMEABILITY TRANSITION AND LIVER APOPTOSIS.

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Emerging evidence indicates that chronic alcohol exposure disrupts the balance between pro- and anti-apoptotic proteins leading to possible enhanced susceptibility to apoptotic cell death via induction of the mitochondrial permeability transition pore (MPTP). The MPTP is comprised of multiple proteins including the voltage dependent anion channel (VDAC), cyclophilin D (CypD), and the adenine nucleotide translocator (ANT). Currently, the mechanism responsible for increased MPTP sensitivity in alcohol exposed mitochondria and it's relation to mitochondrial Ca2+ handling and apoptosis is not known. The aim of this work was to investigate alcohol-dependent susceptibility to the MPTP and mitochondrial Ca2+ accumulation. For this, rats were pair-fed control or alcohol-containing liquid diets, liver mitochondria were isolated. and induction of the MPTP was measured using the swelling assay. Calcium accumulation was measured using Calcium Green 5N. Levels of VDAC, ANT, and CypD were measured by western blot and apoptosis was measured using the TUNEL assay. Liver mitochondria from alcohol-fed animals were more sensitive to calcium-induced MPTP opening than controls. Similarly, mitochondria from alcohol-fed animals accumulated less calcium than control fed animals. We also observed a significant increase in CypD protein in mitochondria from alcohol-fed animals compared to controls, whereas there was no change in ANT and VDAC. Furthermore, we observed an increase in TUNEL positive nuclei in alcohol-fed animals compared to controls. It has been proposed that CypD binding to the ANT is increased by oxidative stress and this interaction facilitates a conformational change in the ANT leading to induction of the MPTP.
Importantly, an alcohol-dependent increase in Cyp3A4 may result in greater susceptibility to MPTP induction contributing to mitochondrial dysfunction, bioenergetic stress, and increased apoptotic cell death in alcoholic liver.

**1634 ROLE OF TGFβ-MEDIATED HEPATOCYTE EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) IN ALCOHOL-INDUCED LIVER FIBROSIS.**

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Hepatic fibrosis, initiated by excessive alcohol consumption, is a predisposing factor in the development of cirrhosis. Excessive production of extracellular matrix (ECM) causes destruction of liver architecture leading to cirrhosis, loss of liver function, and eventually death. Deposition of ECM is stimulated by TGFβ, which is produced and activated during the fibrotic process. While hepatic stellate cells have previously been viewed as the sole source of ECM production, new evidence suggests that other cell types can contribute to hepatic fibrosis. We hypothesize that TGFβ leads to EMT in hepatocytes, which contributes significantly to ECM deposition seen in alcohol-induced liver fibrosis. Using a mouse model of liver fibrosis, 5% ethanol (v/v) administered in a fortified liquid diet for 2 weeks is shown to result in a 2-fold increase in collagen deposition in the liver. In addition, qRT-PCR analysis shows that hepatocytes purified from animals treated with ethanol in vivo had a 2-fold increase TGFβ1, concomitant with a 4- and 8-fold upregulation of COL1A2 and CTGF, respectively, while Snail mRNA was upregulated 5-7-fold, indicative of the process of EMT. Oxidative stress caused by ethanol consumption has previously been shown to cause expansion of hepatic progenitor cells. Analysis of hepatocytes for cell surface markers that are known to be expressed by hepatic progenitor cells showed that ethanol produced a 2.5-fold increase (5.7% to 16%) in CD90 cell surface expression over control levels, and a modest increase (2.7% to 3.7%) in CD44 expression. In addition, we show that the hepatocyte fraction showing high CD90 expression identifies the population which is enriched for CD44, collagen type I, Snail and TGFβ expression. Together these results demonstrate that in vivo ethanol treatment leads to evidence of TGFβ-induced hepatocyte EMT, and suggests that the CD90+ cell population contributes to excess matrix production during ethanol-induced hepatic fibrosis.

**1635 PTEN INHIBITION BY 4-HNE IN HEPG2 CELLS LEADS TO INCREASED AKT ACTIVATION AND CELL SURVIVAL.**

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The production of reactive aldehydes such as 4-hydroxyxenononal (4-HNE) is a key factor in the pathogenesis of alcoholic liver disease. While in the T-cell lymphoma Jurkat cell line 20hM of 4-HNE lead to apoptosis, in both hepatic and the hepatic carcinoma cell line HepG2, exposure to 4-HNE is well tolerated at doses up to 100hM. The mechanism of hepatic cell resistance to 4-HNE has not been well established with respect to the pro-survival kinase Akt and Akt related signaling pathways. In the present work, we examined the effects of 4-HNE on the hepatic carcinoma cell line HepG2 using western blotting and enzymatic activity assays. Treatment of HepG2 cells with 4-HNE resulted in activation of Akt within 30 minutes of cell treatment and at doses above 25hM as shown by increased phosphorylation of residues ser473 and thr389 of Akt. Treatment of cells using both okadaic acid and Ly294002 suggested that activation of Akt is independent of PP2C and Pdln3 kinase-dependent. Using biotin hydrazide modification and Streptavidin pull downs, we observed that the Akt regulator PTEN is modified by 4-HNE following 4-HNE treatment and addition of PTEN resulted in inhibition of lipid phosphatase activity. The activation of Akt by 4-HNE inhibition provides a mechanism for increased reactive aldehyde resistance in hepatic cells. (Supported by NIH grant R37NIH/AA09300)

**1636 HYPOXIA-INDUCIBLE FACTOR-1ALPHA REGULATES EXPRESSION OF GENES IN HYPOXIC HEPATIC STELLATE CELLS IMPORTANT FOR COLLAGEN DEPOSITION AND ANGIOGENESIS.**

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Several studies have shown that regions of hypoxia develop in the liver during chronic injury. Furthermore, it has been demonstrated that hypoxia stimulates release of mediators from hepatic stellate cells (HSCs) that may affect the progression of fibrosis. The mechanism by which hypoxia modulates gene expression in HSCs is not known. Recent studies demonstrated that the hypoxia-activated transcription factor, hypoxia-inducible factor-1α (HIF-1α), is critical for the development of fibrosis. Accordingly, the hypothesis was tested that HIF-1α is activated in HSCs and regulates expression of genes important for HSC activation and liver fibrosis. HSCs were isolated from mice and exposed to hypoxia. HIF-1α and HIF-2α activation were measured, and gene expression analyzed by gene array analysis. To identify genes regulated by HIF-1α, HSCs were isolated from Control and HIF-1α Deficient mice. Exposure of primary mouse HSCs to 0.5% oxygen activated HIF-1α and HIF-2α. mRNA levels of numerous genes were increased in HSCs exposed to 0.5% oxygen, many of which are important for HSC function, angiogenesis, and collagen synthesis. Of the mRNAs increased, Ccr1, Ccr5, macrophage migration inhibitory factor, interleukin-13 receptor α1, prost-4 hydroxylase etα2 (PHD α2) were completely HIF-1α-dependent. Upregulation of VEGF and placential growth factor were partially HIF-1α-dependent and upregulation of angiotatin-like 4 and PHD etα1 were HIF-1α-independent. Results from these studies demonstrate that hypoxia, through activation of HIF-1α, regulates expression of genes that may alter the sensitivity of HSCs to certain activators and chemotaxis, and regulates expression of genes important for angiogenesis and collagen synthesis.

**1637 METABONOMIC ASSESSMENT OF THE OVERNIGHT FAST: A TRANSLATIONAL EVALUATION IN RATS AND HUMANS.**

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The overnight fast is one of the most common experimental manipulations used in both the preclinical and clinical settings. Despite this, the metabolic impact of this manipulation has not been studied in detail particularly with regard to cross-species differences. Normal, healthy volunteers (7M/8F) and rats (5/sex) were fasted for 10-14 hours or 16 hrs, respectively. Pretest (2 hr post meal) samples served as control for the clinical samples while a concurrent group of rats (5/sex) maintained on ad lib feed served as control for the rat. Urine and serum samples were collected at termination of the fast for metabolomic analyses which consisted of NMR analysis of the urine and GC/MS and LC/MS analysis of the serum. Significant (p < 0.05) changes in 13/28 (M) and 17/28 (F) annotated metabolites (AM) were noted in rat urine and in 12/23 AM in human urine (combined sexes). In rat serum, significant changes in 8/48 (M) and 13/48 (F) AM were noted by LCMS and 59/153(M) and 74/153(F) were identified by GCMS. Corresponding values in human serum (combined sexes) were 21/60 by LCMS and 25/68 by GCMS. Urine NMR spectral profiles varied significantly between the species. Serum metabolic changes were characterized by mobilization of serum fatty acids in both species and decreases in conjugated bile acids in humans and variable, but marked, increases in non-conjugated bile acids in rats. These data suggest that fasting has a profound effect on both rat and human metabolic profiles and that the metabolic impact of the overnight fast is appreciably more than 10-14 hour fast in human. Further, frank species differences exit in response to fasting that should be understood in the context of translating data from rat models to potential human exposure where fasting is a part of the experimental paradigm of either species.

**1638 METABONOMIC ASSESSMENT OF RAT LIVER SLICES INCUBATED WITH DICLOFENAC AND LIPS.**

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A non-targeted proton NMR metabolomic evaluation of precision-cut rat liver slices was conducted. Numerous changes in small molecules present in the media were observed, including drug-related metabolites, depletion of media constituents as well as appearance of endogenous components produced by the slices. In the absence of any treatment, the metabolomic analysis indicated that among the more profound changes during culture was depletion of media components histidine and choline over time, which suggests that these may be limiting nutrients in the culture media. It has been reported that in rats, lipopolysaccharide (LPS)-induced inflammation potentiates the toxic effects of diclofenac. We were interested in investigating if a metabolomic approach could provide evidence of this phenomenon in
rat liver slices and how the metabonomic changes compared to standard endpoints of hepatotoxicity. Slices were incubated with 0, 0.5, 1.0, and 2.0 mM diclofenac in the presence and absence of 1, 10, and 100 μg/mL lipopolysaccharide (LPS). Diclofenac metabolism was determined by mass spectrometry and covalent protein binding of 14C-diclofenac confirmed the generation of chemically reactive intermediates, an observation consistent with the known biotransformation properties of this agent. Induction of an inflammatory state by LPS was assessed by monitoring total nitrates/nitrites. There were no discernable metabonomic differences between slides treated with diclofenac and those treated with both diclofenac and LPS, which was in agreement with the AST and ATP analysis. These results suggest that metabonomic evaluation of liver slices can reveal novel biochemical events occurring in slice cultures, and that under the above stated conditions, rat liver slices do not model the in vivo LPS potentiation of diclofenac hepatotoxicity.

**1639 EFFECTS OF DIET INDUCED OBESITY ON TOXICOLOGICAL AND TRANSCRIPTIONAL ENDPOINTS.**

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Diet has profound effects on physiology, disease and lifespan. Diet induced obesity (DIO) models are frequently used to investigate the physiological consequences of excess calorie intake and the sequelae of increased body fat. Since drug efficacy and safety frequently interact with underlying physiology, it is critical to understand the impact of the underlying model on efficacy and toxicity endpoints. This study investigated pathologic and transcriptional differences in obese and lean animals. Male C57Bl/6J mice beginning at 4-weeks of age were fed for 24-weeks a high fat (DIO) or standard diet (Controls). DIO mice had a high incidence (12/12) of marked/severe widespread micro- and macrovesicular steatosis in the liver and mild clinical pathology changes indicating liver damage. Transcriptional analysis of liver and muscle (soleus, gastrocnemius) revealed marked effects in liver and only minor changes in skeletal muscles. Prominent transcriptional effects in liver included a general down-regulation of genes with roles in cholesterol biosynthesis and an increase in lipid metabolism and transport related transcripts. Inflammation-related transcripts and transcripts for genes with roles in tissue remodelling were induced. The hepatic transcript abundance of numerous drug transporters and drug-metabolizing enzymes were affected in DIO livers relative to normal livers highlighting the possibility that diet and/or obesity may significantly affect drug kinetics, metabolism and disposition in both animal models and, potentially, in people. These results indicate metabolically important differences between lean and obese animals. Furthermore, in combined efficacy/toxicity studies conducted in DIO models, some compound-mediated effects may be exacerbated, whereas detection of other abnormalities might be concealed by underlying pathologies in these animals.

**1640 DEVELOPMENT OF AN IN VITRO GENE EXPRESSION SIGNATURE PANEL FOR PREDICTING SKELETAL MUSCLE INJURY IN RAT.**

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Skeletal muscle fiber injury is a sporadic but recurrent event that has been seen both preclinically and clinically (e.g. statin-induced rhabdomyolysis) during drug development. Screening for identification of molecules that may induce muscle injury preclinically and clinically (e.g. statin-induced rhabdomyolysis) during drug development. Based on that work, we sought to develop a gene expression signature to predict skeletal muscle injury that could be applied in an in vitro cell model (the rat H9c2 myotube cell line). The training set consisted of eleven compounds known to induce muscle injury in rats (positive class) and ten negative class compounds, to which H9c2 myotubes were exposed for 24 hr at a normalized concentration for gene expression microarray analysis. Both machine learning algorithm-based and empirical-based approaches, when applied to the H9c2 microarray data set, were able to identify sets of genes that could correctly classify positive and negative compounds for skeletal muscle injury. Two gene signatures derived from this approach consisted of four genes each and had 66-86 % sensitivity and 92-100 % specificity. The performance of the in vitro H9c2 muscle injury gene signatures were then evaluated by testing six known myotoxicants not in the original training set. Analysis revealed that the signatures were able to classify the test compounds as positive for muscle injury with high accuracy, pointing to the feasibility of developing a focused qPCR-based in vitro transcript panel based on only a few genes to screen compounds for skeletal muscle injury risk.

**1641 TOXICOGENOMIC COMPARISON OF RAT STRAINS AND GENDER IN AN ACUTE MODEL OF CISPLATIN INDUCED RENAL INJURY.**

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The rat is often used in discovery phase drug development to assess pharmacologic and toxicologic parameters prior to human dosing. Strain and gender differences, however, may have a profound effect on the synthesis of pharmacology and toxicology studies. The present study utilized global gene expression profiling to compare male and female Han Wistar, Sprague Dawley, and Fisher rat strains in a model of cisplatin induced renal injury. Transcriptomic analysis was performed using the Affymetrix Rat 230 2.0 array on RNA from whole kidneys harvested from animals 24 hours after IP dosing with 5 or 10mg/kg cisplatin. Pearson Correlation using a list of significantly changed genes from all experimental groups shows clustering influenced in order by gender, control vs. cisplatin treatment, and strain. Gene ontology enrichments for statistically significant changed genes indicate effects on processes involved in cell-cycle progression possibly related to the pharmacological effects of cisplatin. Strain and gender transcript differences in response to cisplatin were further characterized by investigating the effects on transporters, xenobiotic metabolism genes and a panel of renal toxicity biomarkers. The ATP-binding cassette transporters, ABCB1 and ABCC2 show gender and strain differences respectively. ABCB1 is induced in males of all strains studied. ABCC2 is induced in both male and female Sprague Dawley and Fisher, but not Han Wistar rats. Genes in the xenobiotic metabolism ontology show consistent induction in EPHX1 across all strains in both males and females, while FMO4 is down-regulated in males only. Strain and gender differences in response to cisplatin were also noted in kidney mRNAs for the Rules Based Medicine renal toxicity biomarker panel. Data from Kim1 shows up regulation in male Han Wistar and Fisher rats only. This analysis showed no up-regulated markers from this panel in female Han Wistar or Fisher rats. These results underscore the importance of understanding the differences among common rat strains for the translation of pre-clinical to clinical outcome.

**1642 IMPACT OF RAT STRAIN AND GENDER ON TOXICOLOGICAL RESPONSE TO DOXORUBICIN.**


The objective of this study was to investigate the impact of rat genetic factors (strain) and gender on differential toxicological responses to a prototypic cardiotoxicant. To achieve this objective, male or female Han Wistar and Sprague Dawley rats received a single IP injection of doxorubicin (10mg/kg or 20mg/kg). Twenty-four hours after dosing, animals were sacrificed and heart tissue, blood, and urine were collected for analysis. Analysis of protein biomarkers of muscle injury suggested a dose-dependent increase in the amount of overall muscle damage following doxorubicin treatment in both strains and genders. Male Han Wistar rats showed the greatest increase in FABP3 and Mdy3 levels while skeletal troponin (cTnI) was increased to higher levels in the Sprague Dawley strain. Toxicogenomic analysis was performed using Affymetrix rat whole genome arrays on RNA isolated from heart tissue. Examination of significant gene changes by hierarchical clustering revealed that gender was the primary differentiating factor between the males and females, with males being more consistent than the genders, females clustered together by strain and males by treatment. Further analysis of gene expression perturbations induced by high dose doxorubicin treatment in the male rats identified a subset of 131 genes that were commonly perturbed in both Han Wistar and Sprague Dawley strains. These common genes were related to pathways associated with cardiac necrosis and cell death, and gene ontology analysis revealed enrichment in terms related to mitochondrial dysfunction, oxidative stress, DNA damage, and apoptosis. Additional investigation into gene expression differences between male Han Wistar and Sprague Dawley following doxorubicin high dose treatment identified Sprague Dawley strain as having a greater number of overall significant gene changes while transcriptomic perturbations related to various cardiotoxicity pathways were more significant in Han Wistar. Taken together, these data suggest that genetic background is a strong determining factor in the response to toxicants and should be considered when interpreting results from rat toxicology studies.
The effects of individual and combined exposure of rats in a 4-week study to dibutyl-phthalate (DBP) and diethylhexyl-phthalate (DEHP) on the metabolic profile (metabolome) in plasma were investigated. The metabolome was determined based on LC-GC-MS-analysis of blood samples, analysing about 230 metabolites. Results were compared with the BASF metabolomics database for mode of action recognition and to study the potential interaction effects of combined exposure to compounds which share similar modes of action. At the high dose level, DEHP induced a more profound metabolome change than DBP and for both compounds the effect was stronger in males than in females. Both compounds could be identified, based on their metabolome pattern as peroxisome proliferators. When dosed at the toxicological NOEL, DBP induced very few and no consistent metabolome changes, indicating that the toxicological NOEL was also a metabolomic NOEL. Minor, but consistent metabolome changes were noted at the toxicological LOEL. The combined exposure of a high dose level DEHP with a LOEL or NOEL dose of DBP resulted in a virtually identical pattern as with high dose DEHP alone, indicating the absence of interaction. The combined exposure to a high dose DEHP and a high dose DBP resulted in a different metabolome pattern than those seen in animals treated with a high dose of both compounds individually. A statistical analysis of these data demonstrated clear interaction, but the extent of interaction at the level of the metabolome was less than linear additivity. In conclusion, metabolomics is a promising tool for the (early) identification and characterization of toxicological modes of action and can potentially be used to quantify and analyse the nature of interaction of combined exposure.

The report on Toxicity Testing in the 21st Century calls for a "pathway-based" approach to risk assessment. The "omics" technologies, especially gene expression profiling, provide data necessary for a better understanding of toxicity pathways. The number of toxicogenomic studies which incorporate dose-response designs is on the rise, and the applicability of such data to risk assessment is also increasing. To improve quantitative assessment of the toxicity pathways perturbed by potentially hazardous chemicals, it is necessary to integrate dose-response information into the pathway analysis. Unfortunately, the existing gene-set testing methods for genomic data are not well-suited to dose-response study designs, where it is desirable to make a global summary of the transcriptional response for each pathway, while preserving appropriate error control. Here, we propose an approach for dose-response analysis of gene expression data at the pathway level, filling a gap in our existing tools. First, a fast dose-response curve fitting procedure is applied to the expression data. This step substantially reduces the computational cost compared to competing procedures and makes re-sampling feasible. Next, the pathways enriched for dose-responsive genes are detected using SAFE, a resampling-based test of categorical significance in gene expression data. Finally, pathway-based dose-response profiles are generated, which can enable estimation of a pathway-based EC50 value, and a bootstrap procedure is used to obtain confidence intervals. This approach extracts invaluable dose-response information from gene expression data, while accounting for variability and uncertainty at the pathway level.
with gender differences in AUC and Cmax, Hmox1 (heme oxygenase) and Itih4 (inter-tc-trypsin inhibitor, heavy chain 4) were among genes demonstrating high levels of overexpression in rat lung. Other consistently over-expressed genes in the lung included Bcl2, cyclin D1 and 10thk upstream; Bmis11 (breast cancer metastasis suppressor); and two cyclin-dependent kinase inhibitors (Cdkn1c and Cdkn3). Conversely, Cdk1 (cyclin dependent kinase), Fgfbp1 (fibroblast growth factor binding protein 1), and Gstm1 (glutathione-S-transferase, mu1) were consistently down-regulated in the lungs of SR-treated rats. Differential expression of any of these genes could underlie the chemopreventive activity of SR. Although these genes demonstrated consistent patterns of differential expression in the lung of treated rats, most were not differentially expressed in liver. Bcl2-like 11 was the only gene studied that was consistently up-regulated in the liver of all groups treated with SR. (Supported by NCI-N01-CN-43304)

A major goal of this project was to establish translational tools to detect altered drug response in humans and apply this to drug safety-related species. Here we use alternative splicing of pre mRNA as the translating technology. Individual mammalian genes often produce multiple mRNA and protein isoforms that may have related, distinct, or even opposing functions. Recent studies show that 92-94% of human genes undergo alternative splicing in a tissue-specific manner, events that play a major role in the regulation of normal and disease-related processes and drug response. By mapping millions of known human transcripts including expressed sequence tags (ESTs) on to assembled genomes, we have created a comprehensive database of alternative splicing events; 28,185 human genes express 239,206 splice isoforms including 75,962 exon skip events, 24,287 intron retention events, 83,568 alternate first/last exons, and 67,256 alternate donor/acceptor sites. Currently, the database defines 30 organisms including human, mouse, rat, dog, and non human primate. To profile the splicing events in our database, oligonucleotide probes were designed that can specifically quantify alternative splice variants of all or subsets of genes such as those associated with ADME-tox. Microarrays containing these probes have been validated and used to profile splicing events in samples from cancer patients (via NIH funding). Differential splicing of many ADME-related genes was detected in members of ABC transporter, cytochrome P450 and solute carrier subfamilies. The comprehensive database of splicing events and the respective oligonucleotide probes are platform-independent and methods from sample processing through informatics analysis have been established and validated. We are currently adapting these methods to next generation sequencing technologies and multiplexing platforms for direct application into freshly collected and biobanked samples from clinical trials and toxicology studies.

**1648 TRANSLATIONAL TOXICOLOGY: NEXTGEN TOOLS TO APPLY ADVERSE HUMAN RESPONSE INFORMATION INTO RELEVANT ANIMAL MODELS. 1. DETECTION OF GENOME-WIDE ALTERNATIVE SPLICING.**

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**1649 GLOBAL CHIP-CHIP ANALYSIS OF AHR ENRICHMENT IN C57BL/6 MOUSE LIVERS.**

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Responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are mediated by the AhR, a ligand-activated transcription factor that binds dioxin response elements (DREs) with the GCGTC core. Global genomic chromatin immunoprecipitation (Chip-chip) and gene expression analysis using the Affymetrix Tiling 2.0 Arrays and Agilent 4/is4k oligonucleotide arrays, respectively, were performed on hepatic tissue from immature ovariectomized mice orally gavaged with 30μg/kg TCDD. Chip-chip analysis using conservative false discovery rate of 1% identified a total of 1446 AhR enriched regions at 2h. 76.5% of these regions were found within the untranslated regions (UTRs), coding and 10thk region upstream of known transcription start sites (TSSs). At 24h, 57/4 AhR interactions were found with only 7.1% in the UTRs, coding and 10thk upstream promoter region. Within the 10thk upstream region, 2801 and 189 interactions were discovered at 2h and 24 h, respectively, with ~50% of those occurring within the first 1.5kb upstream of the TSS, and ~75% in the first 5kb. The enriched regions at 2h and 24h represented 5627 and 770 unique genes, respectively, with 735 genes common to both time points. Computational DRE searches identified 652 genes possessed 2 or more high-scoring putative DRE (matrix similarity score>0.80). Microarray analysis identified 2451 differentially regulated genes (fold change>1.5, p(1)<0.05) at one or more time points with 163 exhibiting AhR enrichment within 10thk of the TSS to the end of the 3rd UTR. Comparative analysis identified 149 genes that had more than 2 high-scoring putative DREs, AhR enrichment and differential gene expression. This study is the first genome wide analysis of AhR-DRE interactions that integrates computational DRE searches and genome wide gene expression analysis that further enhances our understanding of AhR-mediated gene expression that may lead to toxicity. Funded by CIHR MOP-82715 and SBRP P42ES04911.

**1650 TRANSCRIPTOMIC ANALYSIS IN UMBILICAL CORD BLOOD OF CHILDREN EXPOSED TO GENOTOXIC COMPOUNDS THROUGH THE MATERNAL DIET.**

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Over the last decades, an increased incidence of childhood cancers is seen. A risk factor for their development may be exposure to carcinogenic compounds during pregnancy. Molecular epidemiology studies disease causation using biomarkers; gene expression profiling forms a promising tool for the development of new biomarkers. Our objective is to develop novel genomics-based biomarkers in blood for genotoxic risks in newborns within the project NewGeneris. Umbilical cord blood samples were selected from the Norwegian BraMat cohort (IRB protocol followed and approval obtained). Maternal dietary carcinogenic exposure was assessed using food frequency questionnaires. Transcriptomic profiles were generated using miRNA expression profiling analysis, we used Ambion mirVana miRNA array (bisphenol A). In the results, commonly expressed genes were associated with immune response. In the view of miRNA expression profiling, some of the miRNA was down expressed such as, miR-26a, let-7c, miR-373, miR-565, miR-141, miR-195 and miR-34a. Analysis of miRNA-mRNA interaction showed that miR-34a and let-7c target
above genes and they were related activation of GTPase mediate signaling pathway and apoptosis. These results indicate that involvement of miRNA is crucial in regulating gene expression in response to environmental toxic compounds. Therefore, miRNA-miRNA interaction studies are effective and can be advanced to analyze toxicity and toxicogenomics.

**1652 N-NITROSO COMPOUNDS AND COLON CARCINOGENESIS: AN IN VITRO TOXICOGENOMICS APPROACH.**


N-nitroso compounds (NOC) may play a role in human carcinogenesis since many are genotoxic, and known to be carcinogenic in animals. Since the human gastrointestinal tract is subject to endogenous nitrosation, we hypothesize that NOC exposure targets genetic processes relevant in colon carcinogenesis. To investigate genomic responses following 24 hours of NOC exposure, we analyzed the transcriptomic effects of genotoxic concentrations of two nitrosamides, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (MNU), and four nitrosoamides, N-nitrosodiethylamine (NDEA), N-nitrosodimethylamine (NDMA), N-nitrosopiperidine (NPIP), and N-nitrosopyrrolidine (NPPR), in the human colon carcinoma cell line Caco-2 using microarrays. Cell cycle distribution and apoptosis were analyzed by flow cytometry as phenotypic markers of effect. Gene group and pathway analysis revealed that the nitrosamides had little effect on gene expression, whereas nitrosoamides had a strong impact on the transcriptomic profile. Modifications of cell cycle regulation and apoptosis pathways were found for nitrosoamides which was supported by flow cytometric analysis showing cell cycle blocks and increased levels of apoptosis. Additional modifications were found in oxidative stress and inflammation related gene groups and pathways, which suggest an increase in oxidative stress and pro-inflammatory immune response upon nitrosoamide exposure, although less distinct for NDMA. Furthermore, NDEA, NPIP and NPPR most strongly affected several development genes and pathways, which may influence developmental processes. Nitrosoamide exposure had minimal effect which may be due to the unstable nature of this class of NOC resulting in responses earlier than 24 hours. In summary, we have identified nitrosoamide induced gene expression modifications, many of which may influence the human colon cancer risk.

**1653 TOWARDS QUANTITATIVE RISK ASSESSMENT IN SYSTEMS TOXICOLOGY: ACETAMINOPHEN-INDUCED HEPATOTOXICITY AS A CASE.**

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Until now, toxicogenomics studies have primarily been designed for hazard identification of chemical entities in animal models. However, in view of the ambitions expressed in the NAS report "Toxicity Testing in the Twenty-first Century: A Vision and a Strategy", toxicogenomics should be employed to contribute to a systems-based toxicology and thereby to quantitative risk assessment. The first step needed in this evolution is proving the relevance of animal toxicogenomics to humans. Toxicogenomics can aid in the scientific challenge of toxicogenomics to cross-species as well as in vitro to in vivo extrapolation as it enables comparison at a new level of organization, i.e. the molecular level of mRNA and protein. These similar endpoints can be compared using a parallogram approach to further extrapolate to humans. In the current presentation, this approach is explored in the field of hepatotoxicity using acetaminophen (APAP) as a model compound by analyzing a cross-section of studies on APAP-induced changes in toxicity pathways at the transcriptome, proteome, and metabolome level. Integration of these previous data shows similarity of perturbed pathways across species and between in vitro and in vivo, particularly in oxidative stress-mediated impaired energy metabolism and mitochondrial dysfunction. These effects can thus be considered as most relevant for APAP hepatotoxicity in humans. Now that toxicity pathways perturbed upon APAP exposure have been determined, the challenge for quantitative extrapolation between models and species lies in the establishment of tolerance distributions for these specific biological responses.

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**1654 BLOOD BASED TRANSCRIPTOMIC AND METABOLOMOMIC SIGNATURES FOR HUMAN EXPOSURE TO LOW DOSES OF ACETAMINOPHEN.**


The diagnosis and management of drug-induced liver injury (DILI) is hindered by the limited utility of traditional clinical chemistries. We are investigating peripheral blood (PB) transcriptome signatures as new biomarkers of DILI in humans. We used DNA microarrays as well as serum metabolomic methods to characterize changes in the transcriptome and metabolome in serial PB samples obtained from 6 healthy adults treated with a 4 g bolus dose of acetaminophen (APAP) and from 3 receiving placebo. Treatment did not cause liver injury as assessed by traditional liver chemistries. However, 48 hours after exposure, treated subjects showed marked down-regulation of genes involved in oxidative phosphorylation and mitochondrial function that was not observed in the placebo (p<1.66E-19). In addition, we found a clear increase in oxidative stress and pro-inflammatory immune response upon exposure to APAP overdose patients and rats receiving toxic doses of APAP. A larger follow-up study of 48 individuals receiving 1 g APAP dose four times per day for 7 days as well as 11 individuals receiving placebo treatment found a subgroup of 14 subjects with clear increases in serum ALT levels. A number of transcriptomic changes were found that varied with extent of response to the treatment. The goal of this study is to provide PB gene expression signatures or biomarkers of human liver injury that may improve upon current clinical diagnostic tools.

**1655 USE OF LASER CAPTURE MICRODISSECTION AND AFFYMETRIX GENECHIP PROFILING TO DEVELOP A COMPREHENSIVE GENOME EXPRESSION DATABASE FROM MULTIPLE RAT TISSUES.**

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Knowledge of tissue distribution, localization, and species differences in the expression of candidate drug targets can help guide selection of relevant preclinical animal models and provide insight into possible target-related toxicities. While the gold standard methods for measuring target expression are immunohistochemical (IHC) and in situ hybridization (ISH) staining of frozen or fixed tissues, these are not high-throughput approaches that can be further slowed by the need to develop appropriate reagents. To facilitate determination of target expression in preclinical species we previously used Affymetrix GeneChips® to measure gene expression in multiple tissues from rat, dog, and mouse to create a series of "ZooMaps." We report here the development of a second-generation database we call ZooMap 2.0, which is comprised of gene expression profiles of laser captured micro-dissected (LCM) rat tissues. This database allows the rapid determination of gene expression levels in morphologically and functionally distinct regions in multiple tissues. LCM was performed using the PALM MicroBeam system with catapulting into RLT buffer. Following RNA isolation with the Qiagen RNeasy® Micro kit expression profiling data were generated on Affymetrix GeneChips utilizing the Nugen Pico Kit® labeling protocol. A web-based tool was built to query the databases by gene accession number, internal gene identifiers, Affymetrix ID, or by keywords. Analysis of the ZooMap 2.0 data demonstrates that within most tissues the LCM profiles are distinct from each other and from the whole tissue, suggesting cell-specific expression patterns. Successful enrichment by LCM was demonstrated by the high expression of smooth muscle actin in the smooth muscle layers of several organs and the high expression of keratins 18 and 19 in the mucosal layers of the same organs.

**1656 LAMP-2 IMMUNOHISTOCHEMISTRY (IHC) ENHANCES TISSUE DETECTION OF DRUG-INDUCED PHOSPHOLIPIDOSIS IN RATS.**

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Improved methods for phospholipidosis (PLD) detection and screening are necessary to make early, informed choices of candidate compounds to pursue into pharmaceutical development. As part of a 1-month study, male rats were dosed with flu-
oxetine (30 mg/kg reduced to 15 mg/kg) or a trifluoromethyl tricyclic indole (TTI) compound (5 and 50 mg/kg). Rats were necropsied on days 3, 8, 15, and 29 and tissues were collected for assessment by conventional histopathology (H&E), IHC of lysosomal membrane protein LAMP-2 expression, and electron microscopy to determine the time-course of PLD development. H&E assessment identified PLD in cerbellar Purkinje cells, spleen, lung, liver and mesenteric lymph node of fluoxetine-treated animals and in lung and liver of TTI-treated animals. Evaluation by H&E and LAMP-2 IHC indicated PLD with both compounds in lungs as early as day 3. However, at this time, PLD was identified by H&E only in 17% and 33% of the 50 mg/kg TTI and fluoxetine-treated rats respectively, as compared to 66% and 100% by LAMP-2 IHC. In liver, hepatocellular vacuolation interpreted as probable PLD was identified in 17% and 33% of the 50 mg/kg TTI and fluoxetine-treated rats at day 3 by H&E, increasing to 100% in both groups by day 8. However, evaluation of LAMP-2 IHC revealed that the vacuolation was most consistent with steatosis rather than phospholipidosis in both treatment groups. Furthermore, LAMP-2 IHC was able to demonstrate biliary PLD in all fluoxetine-treated animals but one by day 15, a finding not identified in any liver by H&E. These data suggest that LAMP-2 IHC is a useful tool for enhancing both the sensitivity and specificity of tissue evaluations for drug-induced PLD and may also allow earlier identification of PLD-inducing compounds than would be possible using conventional histopathology alone.

**1659** DIFFERENTIAL GENE EXPRESSION PROFILES OF G-PROTEIN COUPLED RECEPTOR (GPCR) SIGNALING PATHWAYS IN THE DUODENAL AND BLADDER TISSUES OF THE RATS TREATED WITH ATROPINE IN VIVO.


Blockage of muscarinic acetylcholine receptors (mAChRs) is known to induce contraction, decreased exocrine secretion, urinary retention, and other effects. However, there are few reports concerning the underlying gene expression profiles of GPCR signaling pathways on targeted organs following acute exposure to anti-mAChR agents such as atropine in animals in vivo. Such analyses could be critical for the establishment of predictive testing systems to identify potential adverse effects caused by testing agents. In this study, we used the Rat GPCR Signaling PathwayFinder™ PCR Array that consisted of 84 genes and evaluated their expression patterns on duodenal and bladder tissues of male SD rats following 3-hour atropine treatment at 20mg/kg, i.e. The results showed that in the rat duodenal tissue, atropine treatment dramatically changed gene expression of the CAMP nucleotide second messenger (CAMPPKA), calcium signaling, protein serine/threonine kinase, and PI-3 kinase pathways, including over 10-fold down-regulated expression of five genes (Ardb, Cdkn1a, Fos, Gprh and Rgs2), and up-regulation of six genes (Ccl2, Cc4h, Cg5f, Gnas, Pgdr, Rgs2), compared to the vehicle-treated animal tissue. Gene expression in the G-protein coupled to IP3 second messenger (PLC) and MAPKKK cascade pathways were not affected. In contrast, in the bladder tissue, the same treatment up-regulated expression of four genes (Bai1, Ccne1, Crr1, and Gm1) by 2-3 fold, compared to controls. In summary, the results suggested that the in vivo gene profiling of the targeted organ and changes in a panel of genes identified in different tissues has utility as a selective and effective earlier response predictor for adverse effects of agents acting on mAChR pathways.

**1660** DISTINGUISHING GENOTOXIC FROM NON-GENOTOXIC COMPOUNDS USING AN 11 GENE RT-QPCR GENE EXPRESSION PROFILE ANALYSIS.

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The utility of gene expression profiling to develop specific biomarker panels is being tested in many fields such as personalized medicine and drug discovery where toxicity is the leading cause of drug candidate attrition. A major advantage of limited content gene panels is the ability to switch detection platforms from hybridization microarrays to more sensitive and faster reverse-transcription real-time PCR (RT-qPCR) arrays. Additionally, since total RNA can be readily accessed from cell cultures used for testing cytotoxicity during the drug discovery process, a panel mRNA assays that would differentiate genotoxic from non-genotoxic mechanisms of toxicity would provide valuable additional information to investigators. A panel of 168 candidate gene assays was selected from DNA damage pathway analysis and published microarray experiments to generate two different RT2 Profiler PCR Arrays. HepG2 cell cultures were treated with varying doses of nine genotoxic (benzo(a)pyrene, benzo(k)fluoranthene, camptothecin, cisplatin, doxorubicin hydrochloride, 5-fluorouracil, mitomycin, mitoxantrone dihydrochloride, methyl methanesulfonate, and N-Nitrosodimethylamine) and ten non-genotoxic compounds, assayed for cell viability and their RNA isolated for expression analysis. The real-time PCR data from the PCR arrays showed distinctive expression pattern differences correlating with compound dose and level of cytotoxicity in the culture. Statistical and cluster analysis of the data further identified an 11 gene subset of the
candidate panel that could be used independently to assess whether a compound was genotoxic or not. With this panel of gene assays as a core, trends in the results indicate that additional analysis using a larger set of training compounds should allow the expansion of this panel to include assays that will further classify different types of genotoxic mechanisms for test compounds.

The traditional method of evaluating carcinogenic activity and chronic toxicity of a specific chemical has been the two-year animal bioassay. Lower cost, higher throughput models are needed. Organotypic 3D liver co-cultures can sustain liver specific function in vitro for months. Chemically induced transcriptional profiles have been derived from these 3D liver co-cultures to provide an in vitro alternative to predict the hepatocarcinogenic potential of compounds in the standard two-year animal bioassay. Microarray data was generated from cultures exposed to 12 toxicants representing five different compound classes to assess the ability of 3D liver to reflect in vivo transcriptional responses. Baseline gene expression analysis indicated that 3D liver cultures, native liver, and primary hepatocytes are distinct transcriptionally from each other when assessed using unsupervised analytical methods. However, the differential regulation of pathways known to be regulated by these compounds was similar between 3D cultures and in vivo liver.

In 3D cultures, PPAR agonists clofibrate and Wy-16463 upregulated genes in the fatty acid beta oxidation pathway; CAR/PXR agonist Phenobarbital upregulated Cyp3A and Cyp2B; and AhR agonist TCDD upregulated Cyp1A1 and other AhR-responsive genes. Inflammatory agents LPS, TNFα, and IL-6 induced an acute phase response in 3D cultures similar to that induced in vivo. 3D treatments were tested with a library of classifiers that can predict a variety of hepatotoxicities and which were derived from in vivo gene expression data. For example, PPARt treatments scored positive against a peroxisome proliferator classifier and, Phenobarbital scored positive against an AhR activation classifier. The functional and transcriptional studies indicate that 3D liver replicates in vivo function.

Inhibition of calcium-independent phospholipase A₂ activates MAP kinase signaling pathways during cytosistasis induced by Ca²⁺-independent phospholipase A₂ (iPLA₂) inhibition were investigated. iPLA₂ inhibition, using the selective inhibitor bromoenoic lactone (BEL) and siRNA, decreased growth in LNCaP (p53 positive) and PC-3 (p53 negative) human prostate cancer cells. In addition, iPLA₂ inhibition induced cell cycle arrest and activated p38 in both cell lines, while ERK1/2 was transiently activated only in PC-3 cells. Inhibition of iPLA₂, also induced p53 and p21 expression in LNCaP cells. Inhibition of p38 using SB202190 or SB203580 inhibited BEL-induced p53 and p21, as well as G1 arrest in LNCaP cells, but had no effect on G2/M arrest in PC-3 cells. In contrast, inhibition of ERK1/2 using PD98059 only slightly altered p38 activation in LNCaP cells, but did alter S-phase arrest in PC-3 cells. BEL treatment also induced reactive oxygen species (ROS) in PC-3 and LNCaP cells, which was reversed by pretreatment with either N-acetyl-cysteine or deferoxamine. These antioxidants also inhibited BEL-induced activation of p38 and p53 in LNCaP cells. On the other hand, treatment of PC-3 cells with the EGF inhibitor AG1478, or the matrix metalloproteinase (MMP) inhibitor GM6001, inhibited BEL-induced ERK1/2 activation, but not p38 activation. Collectively, these data demonstrate that inhibition of iPLA₂ activates p38, ERK1/2 and p53 in correlation with cytostasis. Activation of p53 is mediated by a ROS-p53-dependent pathway, while activation of ERK1/2 is mediated by pathways including EGF and MMPs. Thus, these data demonstrate the novel findings that iPLA₂ inhibition differentially activates p38 and ERK1/2, and further suggest that these signaling kinases have differential roles in cell growth.

The role of HMGB1 in silica-induced inflammation and fibrogenesis in mouse lungs.

Silica-induced oxidative stress at the particle surface is thought to contribute to tissue pathologies. Oxidative stress stimulates the β-catenin signaling pathway which is important in mediating cellular proliferation, a key step in fibrogenesis. When activated, β-catenin translocates into the nucleus where it binds to high mobility group box-1 (HMGB1), a chromosomal protein regulating expression of genes controlling cellular proliferation. Evidence suggests that HMGB1 is also passively involved in the control of c-FLIP expression in testicular germ cells after MEHP-induced Sertoli cell injury.

The activation of mitogen activated protein kinase (MAPK) signaling pathways during cytosistasis induced by Ca²⁺-independent phospholipase A₂ (iPLA₂) inhibition were investigated. iPLA₂ inhibition, using the selective inhibitor bromoenoic lactone (BEL) and siRNA, decreased growth in LNCaP (p53 positive) and PC-3 (p53 negative) human prostate cancer cells. In addition, iPLA₂ inhibition induced cell cycle arrest and activated p38 in both cell lines, while ERK1/2 was transiently activated only in PC-3 cells. Inhibition of iPLA₂, also induced p53 and p21 expression in LNCaP cells. Inhibition of p38 using SB202190 or SB203580 inhibited BEL-induced p53 and p21, as well as G1 arrest in LNCaP cells, but had no effect on G2/M arrest in PC-3 cells. In contrast, inhibition of ERK1/2 using PD98059 only slightly altered p38 activation in LNCaP cells, but did alter S-phase arrest in PC-3 cells. BEL treatment also induced reactive oxygen species (ROS) in PC-3 and LNCaP cells, which was reversed by pretreatment with either N-acetyl-cysteine or deferoxamine. These antioxidants also inhibited BEL-induced activation of p38 and p53 in LNCaP cells. On the other hand, treatment of PC-3 cells with the EGF inhibitor AG1478, or the matrix metalloproteinase (MMP) inhibitor GM6001, inhibited BEL-induced ERK1/2 activation, but not p38 activation. Collectively, these data demonstrate that inhibition of iPLA₂ activates p38, ERK1/2 and p53 in correlation with cytostasis. Activation of p53 is mediated by a ROS-p53-dependent pathway, while activation of ERK1/2 is mediated by pathways including EGF and MMPs. Thus, these data demonstrate the novel findings that iPLA₂ inhibition differentially activates p38 and ERK1/2, and further suggest that these signaling kinases have differential roles in cell growth.
released from necrotic cells where it binds to receptor for advanced glycation end products (RAGE) on macrophages and stimulates inflammatory mediator production. In the present studies, we analyzed the potential role of HMGB1 in silica induced inflammation and fibrogenesis. C57BL/6J mice were treated with silica (1 mg/kg. Min-U-Sil 5) by aspiration. After 7 d, we found that silica administration resulted in increased expression of cyclooxygenase-2 and inducible nitric oxide synthase in the lung, enzymes mediating the production of proinflammatory/cytotoxic prostaglandins and reactive nitrogen species. This was associated with increased expression of HMGB1 in lung and the development of fibrotic lesions. These data suggest that HMGB1 and downstream signaling pathways may be important in both silica-induced inflammation and fibrosis. Supported by NIH grants CA132624, ES004738, ES005022, AR055073, GM034310 and HL074115.

1666 USING MULTIPARAMETRIC HIGH CONTENT IMAGING TO ASSESS MECHANISMS OF CELLULAR STRESS AND TOXICITY.
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Understanding cellular toxicity, including effects on a cell's biochemistry, is key to understanding the undesired side effects that a compound may have. High-content image analysis, coupled with antibody-based staining or engineered cell lines expressing a GFP-tagged protein of interest, serve as a useful tool to help understand the underlying mechanisms of toxicity. In this study, several markers of cellular stress, genotoxicity, apoptosis and general toxicity were evaluated. As models of systemic toxicity, HepG2 (liver) and HK-2 (kidney) cell lines were treated with compound and stained with antibodies against various cellular targets such as p-H2AX, p-ATM, p53, cJun, activated caspase 3 and a marker of cell proliferation. In addition, cell density, morphology and DNA content were also analyzed, providing a multiparametric approach to understanding the underlying mechanisms of toxicity. Compounds screened in 96-well microplates demonstrated a range of responses in these targets for both cell lines. Additional markers were studied in engineered cell lines that stably express GFP-chimeric protein, that when activated, translocate from the cytoplasm to the nucleus of the cell. These stable cell lines, expressing GFP chimeras of ATF6, p53-Hdm2, Rad51, and HIF-1a, were plated into 96-well microplates, treated with compounds for a set period of time, fixed, stained with Hoechst (to identify nuclei) and analyzed with a high content imaging platform. The compounds tested showed varying response for the targets of interest. Overall, the multiparametric approach proved to be a useful tool for studying mechanisms of stress and toxicity. The compounds tested showed cell line- and target-specific profiles for markers of cellular toxicity.

1667 MALDI-MS-BASED DRUG AND PROTEIN IMAGING TO SIMULTANEOUSLY DETERMINE DRUG DISPOSITION AND PROTEIN MODIFICATION IN CELLS.
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Understanding the efficiency of drug delivery to target tissues and mechanism of drug action is pertinent in developing an efficacious therapeutic regimen. Our recent models of renal cell carcinoma (RCC) exhibit increased levels of βECD β-phosphorylation at sites critical for the release of eIF4E, facilitating eIF4E-mediated initiation of cap-dependent translation of proteins that likely contribute to tumor progression. We used MALDI-MS to correlate cellular toxicity and protein modification with drug treatment in renal epithelial cell lines. The βECD phosphorylation treatment resulted in a maximal 30% decrease in cell viability at 2 h. Western data revealed that combination therapy resulted in a more profound decrease in p-ERK1/2, S6, Thr70, and TCR4/746 than with either drug as a single treatment. MALDI-MS data also effectively confirmed drug uptake into cells, revealing 394.35 m/z (erlotinib) and 448.2 m/z (MP470). Moreover, we detected induction of several proteins (8559.4 m/z, 7883.06 m/z and 9153.6 m/z) 1.5 h after drug treatment, the identity of which is currently under investigation. The rapid analysis of intact protein levels can be important in determining drug mechanism of action. Taken together we have demonstrated the value of applying MALDI-TOF imaging techniques to simultaneously visualize differential protein expression and localization of drugs in the same sample. We are extending the utility of these methodologies to analyze nude mice xenograft tumor and human tissues following drug treatment, to unravel signaling pathways and posttranslational modifications to key proteins associated with the progression of RCC (GM070890, ES006604, ES007091, ES106652).

1668 ANNEXINS: AN EARLY RESPONSE TO ENVIRONMENTAL TOXICANTS AND A POTENTIAL NEW BIOMARKER OF TUMORIGENESIS.
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Lower molecular weight polycyclic aromatic hydrocarbons (PAHs) with specific structural features are potent in vitro inhibitors of gap junctional intercellular communication (GJIC), and activators of arachidonic acid (AA) release and nitrogen-activated protein kinases (MAPKs), which are cellular events linked with tumor promotion and other pathways. We previously found that inhibition of GJIC in a rat liver oval-like epithelial cell line (WB-F344) is preceded with the activation of phosphatidylinositol-specific phospholipase C (PC-PLC). We used advanced proteomics techniques (SILAC – Stable Isotopes Labeling with Amino Acids in Cell Culture, ZOOM® isoelectric fractionation, 1-DE, 2-DE and mass spectroscopic identification) to further identify early upstream biochemical signaling events. Among the proteins specifically affected within a 5 min exposure time to 1-MeA, annexins A1, A3 and A5 showed significant responses characterized by their disappearance from plasma membrane, while A2, A4, A6, A7 and A11 were not affected. Immunostaining experiments on annexin A3 (AnxA3) indicated a translocation from the plasma membrane within 30 s of exposure followed by reintegration back into the plasma membrane after 60 min. Translocation of AnxA3 from the plasma membrane is effectively prevented by pre-treatment of the cells with PC-PLC inhibitor, D609. Knock-down of AnxA3 by siRNA did not prevent 1-MeA induced inhibition of GJIC but did stimulate the 1-MeA induced release of AA. We hypothesize that annexins closely interact with phospholipases in the plasma membrane until removed from the membrane in response to 1-MeA. We demonstrated that annexins A1, A3 and A5 are implicated in PC-PLC-regulated early events that regulate the release of lipid derived second messengers. Support: NIEHS grant #R01 ES013268-01A2 to Upham.

1669 CYTOOTOXICITY OF CYCLODEXTRINS: IMPLICATIONS IN CELLULAR CHOLESTEROL LIPID RAFT STUDIES.
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Membrane cholesterol (lipid raft-associated) has been emerging as a pivotal player in cellular signaling cascades. Cyclohextrins, such as methyl-β-cyclohextrin (MCD) and hydroxypropyl cyclodextrin (HPCD), have been widely used as tools to deplete membrane raft-associated cholesterol in cell culture models to study the role of cholesterol lipid rafts in cell signaling cascades. However, the adverse effects of cyclohextrins are not thoroughly established in cell culture models. In order to establish the adverse effects of two well-known cyclohextrins, MCD and HPCD, bovine aortic artery endothelial cell (BAEC) were treated with different concentrations of MCD and HPCD (2% and 5%, wt/vol) for 15-120 min and the loss of membrane cholesterol, cell viability (lactate dehydrogenase release and MTT reduction), cell morphology, protein alterations, changes in phospholipid fatty acid composition, cell replication, and cytoskeletal alterations were determined. The results revealed that both MCD and HPCD caused significant loss of membrane cholesterol, loss of cell viability, altered cell morphology, loss of membrane fatty acids, altered proteins, and induction of actin cytoskeletal rearrangement in BAECs. However MCD caused a greater extent of cytotoxicity as compared to that caused by HPCD under identical conditions. Removal of cholesterol by cyclohextrin (especially MCD) treatment, caused loss of fluidity of the cell membrane and leakage of vital cellular components, and thus led to cytotoxicity and biochemical alteration in ECs. Also, the study offered a safer method of cholesterol removal by HPCD treatment, without extensive loss of cell viability, for studies on the role of lipid rafts in endothelial cell signaling.
1670 CHARACTERIZATION OF A CONSTITUTIVELY NUCLEAR, DOMINANT-NEGATIVE ZEBRAFISH MT1 TRANSCRIPTION FACTOR.

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The MT-1 transcription factor responds to changes in cellular zinc levels caused by zinc exposure or displacement of endogenous zinc by heavy metals or oxygen-related stress. In an effort to develop novel molecular tools, a truncated zebrafish MT-1 isoform lacking all transactivation domains (zfMT1-ΔMTAD) was cloned into an expression vector to create a C-terminal eGFP fusion protein. The dominant-negative capacity of zfMT1-ΔMTAD was indicated by its ability to repress endogenous MT-1 activity in Cos-7 cells by 84% when co-transfected with a metal-responsive luciferase construct. The zfMT1-ΔMTAD was able to repress exogenous mouse MTF-1 activity by 84% when both were co-transfected with a zMT promoter construct into MTF-1 null mouse embryonic fibroblast (MEF) cells. To test the hypothesis that a constitutively nuclear, dominant-negative MTF-1 (zfMT1-Δ1) would be more efficient at inhibiting MTF-1 signaling, site-directed mutagenesis was performed to replace the RGEYT linker motif with TGKEP between Zn-fingers 1 and 2 in the zfMT1-ΔMTAD, as previously characterized with mouse MTF-1 (Li et al, 2006). Fluorescence microscopy of zfMT1-Δ1, zfMT1-ΔMTAD, or zfMT1-MTF1-Δ1 GFP fusion proteins was used to characterize MTF-1 localization in Cos-7 cells in response to zinc treatment. Both zfMT1-Δ1 and zfMT1-ΔMTAD GFP fusion proteins were clearly cytoplasmic in absence of zinc and only localized to the nucleus upon zinc treatment. In contrast, the dnMTF-1 was constitutively localized to the nucleus regardless of zinc treatment. To test the hypothesis that the dnMTF-1 would be more efficient at inhibiting MTF-1 signaling, zfMT1-Δ1 was co-transfected into MEF cells along with either mouse or zebrafish MTF-1 and the zebrafish MT promoter construct. The zfMT1-Δ1 inhibited mouse and zebrafish MTF-1 signaling by 97% and 96% respectively, in contrast to the 84% inhibition by the zfMT1-ΔMTAD. A transgenic zfMT1-Δ1 zebrafish strain is currently being created to characterize the role of MTF-1 in development and response to various metal and non-metal stressors.

1671 PROTEIN TYROSINE PHOSPHATASES, TC-PTP, SHP1, AND SHP2, COOPERATE IN RAPID DEPHOSPHORYLATION OF STAT3 IN SKIN FOLLOWING UVB IRRADIATION.

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Stat3 is dephosphorylated in murine keratinocytes in response to UVB irradiation. Treatment with Na2VO3, deensitized keratinocytes to UVB-induced apoptosis with the recovery of phosphorylated Stat3 protein levels, implying that a protein tyrosine phosphatase (PTP) is involved in this mechanism. In the current work, we report that three PTPs including TC45, the nuclear form of TC-PTP, SHP1, and SHP2 are responsible for rapid dephosphorylation of Stat3 in keratinocytes induced by UVB irradiation. Dephosphorylation of Stat3 was rapidly increased after UVB irradiation of cultured keratinocytes. Knockdown of TC-PTP, SHP1, or SHP2 using RNAi showed that these PTPs are in part responsible for Stat3 dephosphorylation following UVB irradiation, respectively. The level of Stat3 was significantly higher in keratinocytes transfected with TC-PTP, SHP1, or SHP2 siRNA in the presence or absence of UVB compared with keratinocytes transfected with control siRNA. TC45 was mainly localized in the cytoplasm of keratinocytes and translocated from cytoplasm to nucleus upon UVB irradiation. Stat3 dephosphorylation was increased with nuclear translocation of TC45. Further studies revealed that knockdown of all three phosphatases, TC45, SHP1, and SHP2, using RNAi recovered the level of phosphorylated Stat3 upon UVB irradiation. In mouse epidermis, the level of phosphorylated Stat3 was initially decreased, following by a significant increase at later time points after UVB exposure. The expression patterns of Stat3 target genes, such as cyclin D1 and c-Myc, followed the changes in activated Stat3 in response to UVB irradiation. Collectively, these results suggest that three phosphatases, TC45, SHP1, and SHP2, are responsible for UVB-mediated Stat3 dephosphorylation and may serve in part as one of initial protective mechanisms against UV skin carcinogenesis.

1672 INF-γ INDUCTION OF DUOX2 VIA A NOVEL, NON-CANONICAL PATHWAY.

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Production of reactive oxygen species by a NADPH oxidase/dual oxidase (NOX/DUOX) family member is a phylogenetically conserved mechanism of host defense in multiple tissues. We have previously shown that DUOX2 is induced by interferon gamma (INF-γ) and rhinovirus. These observations suggest that DUOX2 functions as part of the respiratory tract host defense against virus infection. The location of DUOX2 in the epithelial surface of human respiratory tract epithelium positions it for exposure to both inhaled and systemically-delivered toxins that may perturb its regulation or function. Therefore, we investigated if INF-γ-mediated DUOX2 expression occurred via the Janus Kinases (JAK)/STAT canonical pathway as a foundation for further mechanistic studies of what appears to be a significant antiviral protein in the human lung. All experiments were carried out intriplicate using a human bronchial epithelial cell line (hBE1) in submerged culture conditions. Cells were pretreated with commercially available inhibitors for JAK or STAT phosphorylation followed by INF-γ treatment. CXCL10 was used as a positive control in our studies. Changes in DUOX2 or CXCL10 transcription were measured using qPCR and western blots were used to verify INF-γ-induced STAT1 phosphorylation. As predicted, INF-γ treatment induced STAT1 protein phosphorylation and induced a substantial increase in CXCL10 mRNA expression, which were significantly attenuated by JAK/STAT inhibitor pretreatment. In contrast, JAK/STAT inhibitors failed to reduce INF-γ-mediated DUOX2 mRNA transcription. ERK2 or PI3 kinase inhibition, two alternative INF-γ-induced signaling pathways, similarly failed to abrogate inducible DUOX2 transcription. These data indicate that treatment of hBE1 cells with INF-γ induces DUOX2 transcription independent of the canonical pathways. The existence of an alternative signal transduction pathway for INF-γ is an unexpected finding with implications not only for the study of DUOX2, but INF-γ signal transduction in general.

1673 DICHLOROACETIC ACID PREVENTS SIMVASTATIN-INDUCED MUSCLE DAMAGE IN THE RAT: SUGGESTING THAT IMPAIRED MITOCHONDRIAL GLUCOSE OXIDATION IS RESPONSIBLE FOR STATIN INDUCED MYOPATHY.

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Rare instances of skeletal muscle damage (myopathy) are associated with statin therapy through a poorly defined mechanism. Administration of high statin doses to rats causes selective necrosis of glycolytic muscle fibres after 10 days of repeated dosing. Recently we reported that the gene for the pyruvate dehydrogenase kinase (PDHK) isornod PDK4 is induced within 4 days of simvastatin administration (Mallinson et al. J Physiol. 2009;587:2193-30). The PDK family inhibit the pyruvate dehydrogenase complex (PDC), which controls mitochondrial glucose oxidation. Here we have used the PDK inhibitor dichloroacetic acid (DCA) to investigate the role of impaired glucose oxidation in statin-induced myopathy. Female Han-Wistar rats (n=9/group) were administered vehicle or 88 mg/kg/day simvastatin for 12 days, or 50 mg/kg/day DCA for 5 days followed by 40 mg/kg/day DCA/88 mg/kg/day simvastatin for 12 days. In rats administered simvastatin alone minimal to mild necrosis was observed in the glycolytic fibre rich gastrocnemius muscles of 4/9 rats and mean plasma creatine kinase (CK) activity was elevated 93 fold relative to the vehicle treated group. In skeletal muscle PDK4 gene expression was induced 5 fold and PDC activity significantly increased. Administration of DCA completely prevented simvastatin induced muscle necrosis and CK release, and PDK4 gene expression and PDC activity were equivalent to the vehicle control group. In conclusion this data strongly suggests that impaired mitochondrial glucose oxidation is an initiating event in statin induced myopathy. As glycolytic fibres contain fewer mitochondria than oxidative fibres, impaired mitochondrial glucose oxidation may also explain the fibre type selectivity of statin myopathy.

1674 TONALIDE- AND GALAXOLIDE-INDUCED CELL DEGENERATION IS ASSOCIATED WITH POLYSOPRENYLATED METHYLATED PROTEIN METHYL ESTERASE (PMPMEASE) INHIBITION.

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BACKGROUNDS: The synthetic fragrances and flavors are persistent organic pollutants that tend to bioaccumulate and eventually cause disease. They are widely used in food, cosmetics and cleaning agents. Aromatic monoterpenoids that are widely used are 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopentdal[g]-2-benzopyran (HHC8, Galaxolide) and 7-acetyl-1,3,4,4,6,6,7,8,8-hexamethylcyclopentdal[g]-2-benzopyran (HHCB, Tonalide) which are produced in excess of 4500 tons per year worldwide. Because of widespread use and discharges from industrial
plants, they are present in surface waters and fish in the US and Europe. Consumption of fish or contaminated water leads to bioaccumulation and toxicity. This study was therefore to test whether Galaxolide and Tonalide toxicity is associated with PMPMEase inhibition. A concentration-dependent inhibition of PMPMEase by HHCB and AHTN was conducted. Their effects on the degradation of cultured cells were also studied. HHCB and AHTN inhibited purified porcine liver PMPMEase with IC50 values of 87 and 38 μM, respectively. The two compounds caused the degradation of human neuroblastoma SH SY5Y, A549 lung cancer and Caco-2 colorectal cancer cells with EC50 values of 26 and 98, 58 and 14, 41 and 41 μM, respectively. These results suggest that the reported neurotoxicity of these compounds may be associated with their inhibition of PMPMEase. While their widespread use poses a real risk for individuals who are predisposed to develop neurodegenerative disorders, potential benefits may exist in their prevention of tumorigenesis in individuals predisposed to cancers given that hyperactive polyisoprenylated proteins are associated with about 30% of cancers.

1675 HEAT SHOCK PROTEIN 70-KDA (HSP70) AS A CRITICAL REGULATOR OF PROTOETOXIC STRESS IN Pancreatic Cancer Models.

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The heat shock response (HSR) is a well known biological response to cellular stress. Heat shock protein 70-kD (Hsp70) is a key player in the HSR and is unique in its capability to both prevent misfolded protein aggregation and catalyze protein refolding into native forms. Hsp70 has been implicated in a variety of disease types, from neurodegeneration to cancer. Recent global gene expression profiles of cancer cells exposed to proteasome inhibitors, a recently developed class of chemotherapeutic agents, have identified Hsp70 as one of the top upregulated genes. This seems to indicate that it may perform an essential coping mechanism in response to the build-up of proteins caused by inhibition of the proteasome. Drug-induced protein stress is a particularly attractive strategy against pancreatic cancer due to the high protein secretory burden of pancreatic cells. We established that the proteasome inhibitor bortezomib (Velcade) strongly upregulates Hsp70 in pancreatic cancer cells using real-time PCR and immuno blot methods. Co-administration of histone deacetylase inhibitors with bortezomib enhanced the upregulation. Furthermore, silencing Hsp70 or chemical inhibition of heat shock factor-1 (HSF1) caused an increase in bortezomib-mediated cytotoxicity. This study concludes that Hsp70 is an important cytoprotective mechanism against proteasome inhibition, and is the first to directly examine this phenomenon in pancreatic cancer. While heat shock protein 90-kDa (Hsp90) chemical inhibitors are already in the clinic, only one study to date has identified a specific chemical inhibitor of Hsp70. Thus, research in this area is noticeably lacking, and could produce useful therapeutic agents and valuable research tools.

1676 PKDELTA PROTEOLYTIC ACTIVATION AS A POTENTIAL EARLY MARKER FOR DRUG-INDUCED MITOCHONDRIAL TOXICITY.

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Mitochondrial dysfunction and oxidative stress are increasingly implicated in clinical drug-induced toxicities. Several drugs, Black Box warnings from the FDA are known to impair mitochondrial function. Drug-induced toxic insults disturb mitochondrial networks and permeabilize their outer mitochondrial membrane leading to activation of caspase-dependent apoptosis. Therefore, identification and validation of a key signaling molecule for drug-induced mitochondrial toxicity will be of great importance in assessing drug safety. Recently, we demonstrated that PKCδ, a novel PKC isoform, is activated at early stages of the mitochondrial dependent apoptotic cascade via a novel mechanism by which caspase-3 proteolytically cleaves to permanently dissociate the catalytic subunit. Following proteolytic cleavage, the persistently active PKCδ catalytic fragment contributes to mitochondrial toxicant-induced apoptotic cell death. Therefore, in this study, we tested the utility of PKCδ proteolytic cleavage in drug induced mitochondrial toxicity. We screened >50 compounds including in the human HepG2 cell model of liver toxicity, including some known mitochondrial toxins such as etoposide, FCCP, oligomycin, and rotenone. HepG2 cells were exposed to test compounds (1-300 μM) over a period of 24 h. Cells were subjected to cytotoxicity and western blot analysis. We found that compounds that induce mitochondrial dysfunction increased PKCδ proteolytic cleavage in a time-dependent manner. Interestingly, the PKCδ proteolytic activation preceded the cell death, suggesting that PKCδ activation is an early event in the drug-induced toxicities. Collectively, these results sug-
1679 INORGANIC ARSENIC EXPOSURE INDUCES A CANCER PHENOTYPE IN KIDNEY STEM/PROGENITOR CELLS IN VITRO.
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Exposure to inorganic arsenic is unequivocally carcinogenic in humans with multiple target sites potentially including the kidney. Recent evidence shows that arsenic is a transplacental carcinogen in rodents and humans. Emerging theory indicates cancer is a disease of stem cells (SCs), and there would be abundant fetal SCs for arsenic to attack during in utero exposures. Therefore, we hypothesized that inorganic arsenic targets SCs, or partially-differentiated progenitor cells (PCs), for oncogenic transformation. Thus, a rat kidney SC/PC cell line, RIMM-18, was continuously exposed to low-level arsenic (500 nM) for up to 30 weeks. Several in vitro oncogenic transformation markers were assessed bi-weekly during arsenite exposure, including matrix metalloproteinase (MMP) activity, colony formation in agar, and cellular invasiveness. Compared to control (0 nM) cells, chronic low-level arsenite-exposed cells showed significantly increased MMP-2 and MMP-9 activity and more rapid proliferation (all >2-fold) by 20 weeks, characteristics typical of cancer cells. In addition, marked increases in colony formation began as early as 6 weeks (~7-fold) and increased invasiveness was seen by 10 weeks of arsenite exposure. Neuroblastoma(CCL-1772), myeloma(HB-174) and neuroblastoma(CCL-147)) and embryonic stem cells and differentiated cells. In embryotoxicity assay, ID50 value (50% Inhibition concentration for genotoxicity) were 0.2, 0.43, 1.5, 15 mM for myeloma, hepatoma, respectively. In cytotoxicity assay, IC50 values (50% Inhibition concentration) were 0.18, 0.48, 0.73, 0.82, 1.3, 3.1, 3.8 mM for myeloma, muscle, neuraloblastoma, NVRQS-EC, fibroblast, NVRQS-ES and hepatoma cell, respectively, and higher than 100nM for the rest of the cells. In embryotoxicity assay, ID50 value (50% Inhibition concentration for differentiation) was 6.0nM, which was classified as strong embryotoxic (classIII). Danofloxacin presented stronger cytotoxicity and genotoxicity on differ-
tediated regulatory mechanisms in hematopoietic stem/progenitor cells (HSC/HPC) will improve our understanding of the origin and progression of diseases ranging from leukemias to autopsia. Previous studies indicated that activation of murine AhR by its potent ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) leads to increased cell numbers in populations enriched for HSC/HPC and compromised constitutive reconstitution of the bone marrow (BM). We hypothesize that AhR regulates HSC/HPC function by altering interactions with their mi-
croenvironment. To test this hypothesis, BM cells were harvested from mice seven days after a single dose of TCDD (30 μg/kg) by gavage. To quantify the numbers of HSCs with long-term reconstitution potential, we injected limiting dilutions (5x10^3-2x10^8) of these treated cells with 10^3 competitive donor cells into BM-ab-
lated hosts. Twenty weeks later, we analyzed the BM’s long-term reconstitution using flow cytometry. No difference was found in the absolute numbers of HSCs between TCDD- and vehicle-treated donor cells. This suggests that the reported compromised HSC reconstitution is not due to different numbers of long-term HSCs. However, TCDD-treated HSC/HPC showed altered migration from ve-
nous circulation to the BM. We further used flow cytometry to measure the levels of proteins involved in migration and enrollement of HSCs to their microenvi-
ronment. In TCDD-treated HSCs/HPCs, we found elevated levels of the chemokine (CXC motif) receptor 4 (CXCR4) and P-selectin glycoprotein ligand 1, and lower levels of the integrin complex formed by CD49d and CD29. CXCR4 is regulated by HIF-1α, PPARγ, and NfκB. The CXCR4 gene also possesses a putative AhR-
responsive element, and responds to TCDD in other tissues. Our data support a novel AhR role in AhR regulation of interactions between HSC/HPC and their microenvironment with consequences on HSC/HPC function. Supported by NIH Grants ES05030, ES05247, and ES07026.
fermentation (quantitative in-cell Western analysis for myosin heavy chain protein normalized by cell number). Both EST and ACDC have similar results with acetic acid (no effects at concentrations <10μM) and 5-fluorouracil (ACDC endpoints; 50% reduction in cell number (EC50 = 0.83±0.06μM and differentiation (ED50 = 0.49μM). With the ACDC assay, bromochloroacetic acid produced effects on differentiation (ED50 = 135±15μM) at a lower dose than required to produce effects on cell number (EC50 = 334±41μM), but the reverse was seen in the EST assay (Bearing cells (E50 = 511±13μM, EC50 = 106±9μM). Differences in EST versus ACDC in the method of cardiomyocyte analysis (presence of beating heart cells/plate versus cardiomyocyte marker/clone), cell stem line (D3 versus J1), and method of differentiation (hanging drop/EV versus adherent culture) may be important in comparing assay results. The ACDC assay is a technique that can be used to evaluate the effects of chemical exposure on differentiation and cell proliferation/death in an embryonic stem cell approach. [This work is approved by EPA but does not necessarily reflect official Agency policy].

1684 EFFECTS OF HALOACETIC ACIDS AND THEIR MAJOR METABOLITES IN A MOUSE EMBRYONIC STEM CELL ADHERENT CELL DIFFERENTIATION AND CYTOTOXICITY (ACDC) ASSAY.


The haloacetic acids (HAAs) are chemical byproducts of drinking water disinfection. Many of the HAAs are developmental toxins when administered to pregnant rodents. Published studies in whole embryo culture (WEC) show that HAAs produce direct effects on rodent embryogenesis. The effects of individual HAAs and their metabolites were evaluated in the murine embryonic stem cell (mESC) ACDC assay.

Chemicals evaluated were: acetic acid (AA); HAAs - iodoacetic (MIA), chloroacetic (MCA), fluorooxycetic (MFO), bromoacetic (MBA), dichloroacetic (DCA), dibromoacetic (DBA), bromochloroacetic (BCA), trichloroacetic (TCA), and trichloroacetic (TBA), bromodichloroacetic (BDCA) and dibromochloroacetic (DBCA) acids; metabolites - glyoxyllic (GOA), oxalic (OXA), glycolic (GCA) acids. A single cell suspension of pluripotent J1 mESC were plated in 96-well plates and maintained under differentiating conditions for 9 days. The concentration dependent effects of each compound on cell number (DRAQ5/Sapphire700 staining) and differentiation (quantitative in-cell Western analysis for myosin heavy chain protein) were determined. The concentration that produced a 50% reduction was calculated for each endpoint and chemical. For cytotoxicity the relative potencies of the compounds were (listed most to least potent): MIA=MBA=BCA=MCA=GOA=MF=TA-TCA=BDCA=BDCA=AA-DCA=GCA, GCA, BDCA and DCA were more potent at altering differentiation than inducing cytotoxicity; MIA, MBA, GOA, MFA were more potent cytotoxicants than at altering differentiation; MCA, DRA, TBA, TCA, TBCA produced effects on both endpoints at similar concentrations. AA and GCA produced no effects at concentrations less than or equal to 10μM. The relative chemical potency for inducing morphological changes in WEC and affecting mESC are similar in the two systems. Thus, many HAAs alter differentiation or perturb cell proliferation or induce excess cell death in mouse embryonic stem cells. [This abstract does not reflect EPA policy.]

1685 EFFECTS OF HALOACETIC ACID MIXTURES IN A MOUSE EMBRYONIC STEM CELL ADHERENT CELL DIFFERENTIATION AND CYTOTOXICITY (ACDC) ASSAY.


The haloacetic acids (HAAs) are chemical byproducts of drinking water disinfection. Source water characteristics (such as bromide level) affects which HAAs are produced. The molar ratio of these compounds were (listed most to least potent): MIA=GOA=MCA=DBA=BDCA=BCA=BDCA:MBA:DBA:BCA:BDCA:BDC in LBM 21:120:90:0:0:0:2:1:9:0:5; in MBM 14:60:32:8:43:1:11:61:40:49; in HBM 2:12:9:24:12:206:49:30:12:3. A single-cell suspension of J1 mESC were plated in 96-well plates and maintained under differentiating conditions for 9 days. The concentration dependent effects of each mixture on cell number (DRAQ5/Sapphire700 staining) and differentiation (quantitative in-cell Western analysis for myosin heavy chain protein) were determined. The concentration that produced a 50% reduction was calculated for each endpoint and reported for each mixture. LBM produced effects on differentiation at 3800μM and altered cell number at 8700μM. MBM was produced effects on cell number 950μM, but did not alter differentiation at the highest testable level (≥6520μM). HBM produced equal effects on differentiation (45μM) and cell number (450μM). HBM and MBM were 10-20 times more potent than LBM for cytotoxicity. However, HBM did not alter differentiation at concentrations that were testable because of the cytotoxicity. The relative potency of the mixtures is similar to the values reported for whole embryo culture. [This abstract does not reflect EPA policy.]

1686 EFFECTS OF ACUTE EXPOSURE OF LI, MN, NI, AND PB ON CELL PROLIFERATION AND DNA REPAIR GENE EXPRESSION IN DIFFERENTIATING MOUSE EMBRYONIC STEM CELLS.

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Environmental exposure to metals has been implicated in the development of a variety of pathologies, especially in cancer induction and abnormal embryogenesis. To understand the influence of metals on embryogenesis, we exposed murine embryonic stem cell (mES) cells to metals during various stages of differentiation to four metals while monitoring cell viability and DNA repair gene expression. Undifferentiated pluripotent mES cells resemble early embryonic stages of mammalian development. In addition, there differentiation status is manipulated by the presence or absence of leukemia inhibitory factor and monitored by the presence of multilayered aggregates (embryoid-like bodies in suspension culture). Exposure of mES cells for 1 hr to increasing concentrations of metals induces cell proliferation (MTT assay), while Rad118, Oggl, and Top3α DNA repair gene expression, in general, decreases. The decrease in gene expression correlates with mutational events, thus suggesting that alteration of DNA repair mechanisms in the presence of acute metal exposure contributes to a variety of genotoxic events. Moreover, cellular response depends on the degree of differentiation of the cell cultures. That is, undifferentiated cells exhibit an induction response (stimulation of cell proliferation) while differentiated cells display an adaptive response (traditional cytotoxic, lacking cell plasticity) for the same exposure parameters. Overall, growth and differentiation properties of the mES cell line mimics early embryonic stages of development, offering novel in vitro characteristics for detecting genotoxic events.

1687 INHALED ACROLEIN DECREASED CIRCULATING ENDOTHelial PROGENITOR CELLS AND THEIR RECRUITMENT IN MICE.

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Cigarette smoke (CS) exposure induces endothelial dysfunction and impairs circulating endothelial progenitor cells (EPCs). The constituent(s) of CS and mechanism(s) responsible for these changes, however, are unknown. Because acrolein, an unsaturated aldehyde, is present at high levels in CS, the effect of acrolein on EPCs and endothelial function was investigated. Adult male C57BL/6 mice were exposed to filtered air or acrolein (0.5, 1, 5 ppm; 2h-4d), and the EPCs in blood (PB) were measured as Flk1+Sca-1+ cells by FACS. Acute exposure to acrolein (5 ppm, 6h) or 4 day (1 ppm) significantly decreased (~42%) PB-EPCs (air: 8.9±1.7 cells per μl; acrolein: 4.9±0.5 cells per μl, n=4, p<0.05). Although 5 ppm acrolein induced modest aortic endothelial dysfunction, no endothelial dysfunction was observed following 4d acrolein at 1 ppm. Because PB-EPCs derive in part from bone marrow (BM), BM-monomonuclear cells were isolated and cultured on fibronectin-coated slides. BM-EPCs from acrolein-exposed mice (1 or 5 ppm) formed more acetylated LDL- and lectin-stained colonies in culture than BM cells from air-exposed mice (air: 7±1 colonies; acrolein: 14±1 colonies, p<0.001). To test if the acrolein-induced decrease in PB-EPCs was due to a failure to recruit BM-EPCs, combined VEGF (100 μg/kg ip.) and AMD3100 (5 mg/kg ip.) treatment was used to increase PB-EPC in air-exposed mice yet failed to increase PB-EPC in acrolein-exposed mice (air+VEGF/AMD3100: 5.8±0.2 cells per μl; acrolein+VEGF/AMD3100: 2.7±1.0 cells per μl, n=4, p<0.05). These data indicate that PB- and BM-EPCs were sensitive targets of inhaled acrolein at levels encountered in CS. Acrolein-induced depletion of circulating EPCs is likely due in part to impaired EPC mobilization and recruitment. Suppression of circulating EPCs could contribute to endothelial dysfunction and increased cardiovascular disease risk of CS exposure.

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1688 THE ROLE OF THE ARYL HYDROCARBON RECEPTOR IN PREGNANCY IMMUNOLOGY.

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The placenta is an important organ for providing nutrients to the growing fetus and also serves as a barrier warding off harmful substances from maternal circulation. The placenta is an organ known to express significant levels of the aryl hydrocarbon receptor (AhR). The role of the placental AhR however is not clear. In mammalian development, trophoblast stem cells give rise to placental tissues protecting fetal allograft in part through modulating the immune response with products like Fas-lig (Fas-L) and Indolamine 2,3-dioxygenase (IDO). In order to investigate the role of AhR in the protective barrier provided by the placenta, we focused on the in vitro response of trophoblast stem (TS) cell to tetrachlorodibenzo-p-dioxin (TCDD) and benzo-a-pyrene (BaP) exposure. TS cells cultures were shown to contain AhR, and AhR then increase their expression of Fas-L when exposed to TCDD. Later when the TS cells were differentiated into a culture enriched with spongiotrophoblast, CYP1A1 enzyme levels increased in response to TCDD exposure. These findings strongly support the presence of active AhR/CYP1A1 pathway in trophoblast pregnancy immunology. (This work was partially supported by NIH ES 09859)

1689 TRANSCRIPTOMICS-BASED IDENTIFICATION OF DEVELOPMENTAL TOXICANTS BY THEIR MODULATION OF EARLY EMBRYONIC STEM CELL DIFFERENTIATION.

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The embryonic stem cell test was designed to predict developmental toxicity based on the inhibition of differentiation in culture of embryonic stem cells (ESC) into cardiomyocytes. We hypothesized that the sensitivity of this model may be improved by the analysis of differentiation-related gene expression changes. Therefore, we studied the gene expression dynamics of ESC differentiation and the effects of embryotoxicants thereupon. ESC were aggregated and then cultured in suspension culture from day 3 onwards. At day 5, aggregates were plated on tissue culture dishes, and differentiation was assessed by evaluation of contracting cardiomyocyte foci at day 10. Cells were exposed from day 3 onwards to embryotoxicants or solvent only. RNA of solvent-exposed cultures was collected after 0, 24 and 48 hours of exposure, and RNA of toxicant exposed cultures was collected after 24 hours of exposure. Samples were hybridized to Affymetrix geneset chips. We selected all genes which were both regulated by the differentiation process and by exposure to at least one of five embryotoxicants tested. Using this set of genes, principal component analysis (PCA) aligned the 0, 24, and 48 hour differentiation cultures in chronological order, which could be considered as the representation of normal differentiation track. Exposed samples showed a significant deviation from this differentiation track, whereas the negative control did not deviate. Moreover, applying this approach to another independent dataset also resulted in identification of embryotoxic compounds. These results show that, using the differentiation track of ESC differentiation cultures, differentiation-modulating effects of diverse embryotoxicants can be identified with a PCA.

1690 ASSESSMENT OF THE MOUSE EMBRYONIC STEM CELL TEST AND PROPOSAL OF PREDICTION PROCEDURE.


An in vitro teratogenicity test with embryonic stem (ES) cells is noteworthy because the test can be used to detect species difference in teratogenicity of a chemical since human and animal ES cells are available. We assessed the ECVM’s Embryonic Stem Cell Test (EST) with 17 commercial products (Hydroxyurea, Boric acid, Penicillin G, etc) used in the ECVM validation and 32 in-house chemicals of known in vivo activity. The assay with our compounds had high false positive rate. All chemicals tested including non-developmental toxic in vivo were classified as embryo toxic. To clarify the characteristic of EST, we analyzed the results obtained from our assay. The concentrations (IC50 and ID50) inhibiting cell (3T3 or ES) growth or ES cell differentiation by 50% of the control level were close among individual chemicals, while there were some differences among the tested chemicals. There was a tendency that chemicals which affect ES or 3T3 cell growth are classified as embryo toxic. This is probably because strong cell growth inhibitors are used as positive controls and inert chemicals as negative in the EST validation studies. Pharmaceuticals or plant protection products are physiologically active. High exposure to these chemicals could affect cell growth and result in being classified as positive. Similar data have been reported by different investigators. We proposed an alternative prediction procedure based on biological significance of IC50/IC50ES, IC50ES and ID50. We assume that IC50/IC50ES, IC50ES and ID50 are surrogates for maternal toxicity, embryonic growth and morphological abnormalities, respectively. Chemicals, which have ID50 lower than IC50/IC50ES, are classified as positive. Our prediction procedure increased accuracy. Because the assay has a significant false-positive rate, careful investigations on whether or not the positive result with human ES cells suggests that the chemical affect development of human embryo are necessary.

1691 ETHANOL CAUSES DISREGULATION OF MOUSE EMBRYONIC STEM CELL DEVELOPMENT.

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Exposure to ethanol (EtOH) has been shown to disrupt gene expression and development of the preimplantation embryo. In this study, mouse embryonic stem cells (mESC) were used to further explore the effects of EtOH on early development. Cultures were established with undifferentiated mESC and propagated without feeder cells but with leukemia inhibitory factor (LIF), to maintain pluripotency, and EtOH at concentrations from 0% through 1.5%. After 10 days, an MTT reduction assay was used to detect EtOH-induced cytotoxicity. Relative to control cultures, EtOH slightly enhanced mESC viability at concentrations of 0.5%-1.0% (not significant), whereas higher concentrations (1.25%) significantly reduced mESC viability by up to 70% (p<0.002). Nanog and Ocry gene expression is upregulated in undifferentiated mESC, but both genes are downregulated when mES cells are allowed to differentiate in the absence of LIF. Expression of Nanog and Ocry in mESC maintained with LIF showed no significant change after 48 hours of EtOH exposure at up to 1.0%. These results suggest that EtOH does not trigger differentiation of undifferentiated mESC. Therefore, we examined the effects of EtOH on differentiation of mESC (in the absence of LIF) into cardiomyocytes over the course of 11 days. Cultures were established with undifferentiated mESC using the hanging drop method in the absence or presence of EtOH at up to 1.5%. Expression of myosin heavy chain (MHC) was used as a marker of differentiation as detected by flow cytometry. After seven days, 2.0% of control cells expressed MHC compared to 1.1% MHC expression in cells exposed to 0.25% EtOH. Expression of MHC was further declined in a concentration-dependent manner to undetectable levels at 1.0% EtOH and higher. After 11 days, 2.6% of control cells expressed MHC whereas cells exposed to EtOH demonstrated reduced MHC expression in a concentration-dependent manner similar to that seen after seven days of exposure. These results suggest that EtOH exposure modifies the development of mESC into cardiomyocytes.

1692 UPTAKE OF GOLD NANO PARTICLES IN MURINE MACROPHAGE CELLS OCCURS WITHOUT CYTOTOXICITY OR PRODUCTION OF PRO-INFLAMMATORY MEDIATORS.

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Gold nanoparticles (AuNPs) show promise for biomedical imaging and diagnostic applications; however, important information on the biological responses of nanoparticles is needed to allow the FDA to evaluate the safety of these materials for clinical applications. The objective of the present study was to investigate the cytotoxicity, inflammatory potential, and cell uptake of AuNPs in cultured RAW264.7 murine macrophage cells in vitro. Macrophages were exposed to AuNPs (60-nm diam., NIST standard reference material) for 24 and 48 hr at concentrations of 0.1-100 μg/ml in the presence or absence of lipopolysaccharide (positive
Nanotechnology is an emerging interdisciplinary science with broad applications in material science, electronics, optics, medicine, and energy. Engineered nanomaterials display electronic, photonic, and catalytic properties unique from their corresponding bulk materials. When functionalized with highly selective biomolecules, the bioactive nanostructures have promising applications in diverse areas such as sensor technologies, drug delivery, antifouling, and wound healing. Some nanoparticles are known to have detrimental effects on various cell types, necessitating bio-effect characterization of nanostructures prior to developing their intended beneficial effect. The goal of this project was to determine if internalized gold nanoparticles affect cell viability and cellular signal transduction in PC12 cells. PC12 cells were cultured with gold nanoparticles of 4, 10, 30, or 60 nm diameters at 1, 5, or 10 micrograms per mL. The MTS mitochondrial activity assay was used to assess cell viability. Effects on cellular signal transduction were assessed via EGFR activation of the EGF receptor. The results demonstrated that the smaller 0.8 nm and 1.5 nm Au NPs were toxic in a concentration-dependent manner, regardless of charge. Gene expression studies showed that Au NPs induced DNA damage and down-regulated the DNA repair mechanism, with the genes varying based on charge. Additionally, there was a significant amount of p53 nuclear localization and caspase-3 expression in cells treated with the positive and negative particles. In comparison, the neutral particles caused minimal caspase-3 expression and an increase in cytoplasmic p53 expression indicating that p53 was being shuttled out of the nucleus to prevent apoptosis. Taken together, these results indicate that nanoparticle surface charge impacts molecular events in the cell with apoptosis as the mechanism of cell death for the charged nanoparticles and necrosis for the neutral particles.

Gold nanoparticles (Au NPs) are currently being used in a wide variety of applications, yet not much is known about their impact on human health. The present study evaluated the role of surface charge of well-characterized gold nanoparticles in producing biological effects. The nanoparticles in this study were comprised of metallic gold cores (0.8nm, 1.5nm and 10nm) stabilized by an organic ligand shell to make the nanoparticle anionic, cationic or neutral. Cellular morphology, changes in mitochondrial function and changes in gene expression specific for DNA damage were evaluated, along with p53 and caspase-3 expression. The results demonstrated that the smaller 0.8nm and 1.5nm Au NP’s were toxic in a concentration dependent manner, regardless of charge. Gene expression studies showed that Au NPs induced DNA damage and down-regulated the DNA repair mechanism, with the genes varying based on charge. Additionally, there was a significant amount of p53 nuclear localization and caspase-3 expression in cells treated with the positive and negative particles. In comparison, the neutral particles caused minimal caspase-3 expression and an increase in cytoplasmic p53 expression indicating that p53 was being shuttled out of the nucleus to prevent apoptosis. Taken together, these results indicate that nanoparticle surface charge impacts molecular events in the cell with apoptosis as the mechanism of cell death for the charged nanoparticles and necrosis for the neutral particles.

Metal nanoparticles (NPs) are being examined as biomedical probes for applications such as purification, sensing, imaging, and drug delivery and traditional studies have focused on the decrease in viability that may occur after exposure to NPs. However, some NPs actually stimulate cell proliferation following exposure. Epidermal Growth Factor (EGF) signaling is known to be an important mechanism in wound healing and has been shown to accelerate the wound healing process. Activation of EGF can also inhibit apoptotic proteins and prevent apoptosis. Over-expression of EGF receptor (EGFR) however, is a hallmark of cancer; therefore an overstimulation of cell proliferation could be cause for concern. The present study evaluated the stimulatory effect seen in human keratinocyte cell proliferation following a 24h exposure to gold nanoparticles (Au NPs). Changes in phosphorylation levels of EGFR were examined at two different tyrosine residues (1068 and 1173) to determine if Au NPs regulated EGF activation. Results showed an increase in the amount of phosphorylated EGFR at residue 1068 but not at 1173 when cells were treated with EGF and Au NPs. This effect was not seen in the nanoparticle alone treatments. The tyrosine residue 1068 has been shown to activate the Erk pathway; whereas, the 1173 residue prevents activation and negatively regulates the EGFR receptor. The increased levels of 1068 phosphorylation, with little to no activation at residue 1173, indicated that the Au NPs potentially facilitate EGF activation of the EGFR receptor.
1698 CHARACTERIZATION OF THE INTERACTION BETWEEN DNA AND ENGINEERED SILICA NANOMATERIALS.

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The development of biocompatible vehicles for specific targeted delivery of therapeutics agents is an area of intense research in nanotechnology. Often, such research overlooks the possibility that these vehicles, being on the same scale as many biological molecules, may unintentionally interact with host DNA. The primary aim of this study is to determine how particles being designed specifically for drug delivery interact with DNA. We have successfully designed a system in which fluorescently-labelled nanoparticles can be used to assess the interaction of nanoparticles with DNA and other biological tissues. The nanoparticles used in this study are amine-functionalized spherical silica particles, 40 and 80 nm in diameter, that are well dispersed and uniform in size. They are manufactured at the Institute of NanoBioTechnology at the Johns Hopkins University and are well-characterized in terms of particle number per volume, particle size and shape, mass, and aggregation. In this study, linearized plasmid DNA was incubated with various concentrations of NPs for 24 hours. The incubations were loaded onto agarose gels and imaged using a fluorescent imager. The results indicate that NPs interact with DNA in a dose-dependent manner and that this relationship can be modelled using standard receptor-binding curves. The experimental described here provides information relevant to the use of nanoparticles as therapeutic and diagnostic agents in medicine. In addition, it may be relevant to developing biomarkers of nanoparticle exposure among workers and others exposed to nanomaterials used for other purposes.

1699 SILVER NANOPARTICLES INDUCED TOXICITY IN CAENORHABDITIS ELEGANS THROUGH P38 MAPK MEDIATED OXIDATIVE STRESS: FUNCTIONAL GENOMICS APPROACH.


In this study, the toxicity mechanism of silver nanoparticles (AgNPs) was investigated in Caenorhabditis elegans using functional genomics approaches. The involvement of the oxidative stress in the reproduction failure observed in wildtype C.elegans to AgNPs exposure was compared with that of loss-of-function mutants of stress response genes. Following to comparative susceptibility study, the upstream signaling mechanism responsible for regulating oxidative stress was studied using the mitogen-activated protein kinase (MAPK) cascades, to understand underlying mechanism of AgNPs-induced oxidative stress. Special focus was made on the AgNPs-induced alteration of C.elegans p38 MAPK signaling pathway; however, the downstream genes known to be regulated by MAPK signaling pathway were also investigated in AgNPs exposed wildtype and mutants C.elegans. Overall results suggest that AgNPs may not directly produce reactive oxygen species (ROS) in C.elegans, however, whether directly or indirectly, oxidative stress seems to be an important toxic mechanism of reproduction failure induced by AgNPs in C.elegans. As a mechanism of toxicity, pmk-1, a p38 MAPK plays an important role in defense process to oxidative stress induced by AgNPs in C.elegans. This study also suggests that C. elegans functional genomics approach seems to be a promising tool for the study of toxicity mechanisms in ecotoxicological research. Acknowledgment: This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2009-0072720).

1700 INVESTIGATION ON DNA DAMAGE, CELL CYCLE ARREST AND P38 MAPK ACTIVATION AS MECHANISMS OF SILVER NANOPARTICLES TOXICITY IN HUMAN LYMPHOMA CELL, JURKAT.


Toxicity of silver nanoparticles (AgNPs) and silver ions (Ag ions) were compared in human cell lines. Among 6 tested cell lines, cell viability of Jurkat cells decreased significantly by AgNPs, whereas, not by Ag ion. Similarly, apoptosis was observed by AgNPs, whereas, not by Ag ions. To investigate underlying mechanism on this different sensitivity of Jurkat cells to AgNPs and Ag ion exposure, an in vitro toxicity assay was conducted focusing on the involvement of the oxidative stress responding signal transduction pathway and transcription factors in the toxicity of AgNPs. The upstream signaling mechanism responsible for regulating oxidative stress was studied focusing on the mitogen-activated protein (MAP) kinase cascade. Three groups of well-characterized MAP kinase cascades, extracellular signal-regulating kinase (ERK), p38 and c-Jun N-terminal kinase (JNK), were investigated. Redox-sensitive transcription factors, such as nuclear factor-kappaB (NF-κB) and nuclear factor-E2-related factor-2 (Nrf-2), were investigated as target transcription factors of AgNPs toxicities. Antioxidant enzyme activities were also investigated. Overall, our results suggest that AgNPs may exert their toxicity in Jurkat cells through oxidative stress and cell cycle arrest. They cause a significant increase in cellular ROS concentrations, and subsequently lead to an induction of p38 signaling pathway. The tested oxidative stress and cell cycle parameters in this study were rather limited in terms of allowing a full understanding of the oxidative stress and cellular response due to exposure to AgNPs in Jurkat cells. Further studies using mRNA and miRNA expression profiling analysis are warranted to better understand the AgNPs-induced cytotoxicity in Jurkat cells. This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MEST) 2009-0084685.

1701 MODULATION OF AN ASTHMATIC RESPONSE BY NANOPARTICLES IN A MOUSE MODEL OF CHEMICAL-INDUCED ASTHMA.


Occupational asthma is a major respiratory health concern. The expanding nanotechnology industries impose an increased occupational risk. The aim of this study was to investigate the modulation of an asthmatic response by titanium dioxide (TiO2) and gold (Au) nanoparticles (NPs) in a murine model of chemical-induced asthma. On days 1 an 8, BALb/c mice were dually treated with 0.3% toluen diisocyanate (TDI) or the vehicle acetone-oil (AOO). On day 14, the mice were oropharyngeally exposed to 40 μL NP suspensions (0.8 mg/kg TiO2 and Au) or sodium nitrate (2.5 mM, vehicle). One day later, the mice received an oropharyngeal challenge with 0.01% TDI. On day 16, the airway hyperreactivity (AHR) was measured. Subsequently, bronchoalveolar lavage (BAL) cell counts, lung histology and total serum IgE were assessed. Compared to non NP exposed TDI-treated mice, a 2- and 3-fold increase in AHR was induced by TiO2 and Au NP exposure, respectively. Similarly, a 3- to 5-fold increase in BAL total cell counts, mainly comprising neutrophils and macrophages, was observed after NPs administration in TDI-treated mice. Histological analysis revealed increased oedema, epithelial shedding and inflammation in the NPs exposed TDI-treated mice. Total serum IgE levels were increased in sensitized animals but were not modulated by NPs. In conclusion, these results show that low dose TiO2 and Au NPs can modulate pulmonary inflammation and AHR in a mouse model of chemical-induced asthma. Acknowledgments: supported by ANR grants n° 05 9 9-05 SET 024-01, n° 06 SEST 24-01, CAMPIL, Legi Poix, the Interuniversity Attraction Pole Program (P6/35) and the Research Foundation - Flanders (FWO G.0547.08).

1702 SILVER NANOPARTICLE TOXICITY IN SKIN CELLS AND EFICACY IN BACTERIA.

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Silver nanoparticles (Ag-nps) are commonly used as antibacterial coatings, personal sanitizing sprays, infant pacifiers and health supplements. The objective of this study was to evaluate the toxicity and antibacterial efficacy of Ag-nps that varied in size, surface condition, and synthesis method against human epidermal keratinocytes (HEK), Escherichia coli, Ag-Resistant Escherichia coli, Staphylococcus aureus, Methicillin-Resistant Staphylococcus aureus (MRSA), and Salmotella sp. Ag-nps samples were either synthesized by base reduction and used either newly synthesized or after 20 washes ("unwashed") 20 nm, 50 nm, 80 nm; "washed" 20 nm, 50 nm, 80 nm, or by laser ablation stabilized with carbon ("carbon-coated") 25 nm, 35 nm. For the bacterial studies, silver nitrate was used as a control for Ag ions. HEK were treated with each Ag-nps sample at concentrations ranging from 1.7μg/ml to 0.000544μg/ml. HEK viability was assessed by MTT, alamarBlue and Celltiter 96 Aqueous One. Bacterial strains were treated with each of the Ag-nps samples using the microbroth dilution method at concentrations ranging from 512
to 0.5 μg/ml. Minimal inhibitory concentrations were similar for each Ag-np across all bacterial strains. Each of the unwashed Ag-nps were found to be toxic to HEK and all bacterial strains between 1-8 μg/ml. Each of the washed Ag-nps and the carbon-coated Ag-nps were found to be nontoxic to HEK and toxic to all bacterial strains between 64-512 μg/ml. Ag-Resistant E. coli died between 2-8 μg/ml only when treated with freshly synthesized Ag-nps, indicating a non-Ag component to the solution. Transmission electron microscopy was utilized to visualize interactions between Ag-nps and bacteria. (Supported by the USAFOSR FA9550-08-1-0182)

### 1703 GENOTOXICITY OF NANOSILVER IN MOUSE LYMPHOMA CELLS.

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Nanosilver has a variety of antimicrobial uses and therefore it is important to understand any potential mutagenicity. In this study, the genotoxicity of nanosilver was evaluated using the mouse lymphoma assay. The single-cell gel electrophoresis assay (comet assay) and gene expression analysis were used to provide information as to the mode of action for the mutation induction. 15,178/97F mouse lymphoma cells were treated with different doses of 5-nm silver particles up to 6 ng/μl. The treatment resulted in a dose-dependent mutation induction. The mean mutation frequency for treatment with 5 ng/μl nanosilver was about 7-fold higher than the untreated control (426 x 10^-6 vs. 60 x 10^-6). The comet assay was performed with a 4 hour-treatment using similar concentrations as those for the gene mutation assay. No induction of DNA damage was found for the nanosilver treatment in the normal comet assay. Oxidative DNA damage, however, was induced by the treatment in a dose-response manner when formamidopyrimidine DNA glycosylase (FPG) was introduced in the assay. Gene expression analysis using oxidative stress and antioxidant defense PCR array (SABiosciences) showed that expression of 15 out of the 84 genes on the arrays were altered in the cells treated with the nanosilver in a concentration of 5 ng/μl. These genes are involved in production of reactive oxygen species, oxidative stress response, antioxidants and oxygen transporters. The results suggest that the 5-nm silver particles are mutagenic in mouse lymphoma cells, and the molecular mechanisms for the mutation induction may result from the oxidative stress produced by the nanosilver.

### 1704 REACTIVE OXYGEN SPECIES AND GENE EXPRESSION ALTERATIONS IN THE HUMAN EPITHELIAL CELL LINE A549 AFTER EXPOSURE TO SILVER NANOPARTICLES IN VITRO.

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Ag nanoparticles (NPs) are being used extensively in consumer products, thus it is important to explore if NPs exhibit any toxicity. In the present study, several parameters of toxicity were investigated in the human lung cell line A549, e.g., induction of reactive oxygen species (ROS), cytotoxicity, gene expression and formation of bulky DNA adducts. PVP-coated Ag NPs were characterized by TEM (69 nm, aspect ratio 1.2), DLS (118 nm) and PW-XRD (78.1 nm). Cellular uptake of the Ag NPs was estimated by atomic absorption spectroscopy (AAS) and flow cytometry. Ag NPs are being used extensively in consumer products, thus it is important to understand any potential mutagenicity. In this study, the genotoxicity of nanosilver was evaluated using the mouse lymphoma assay. The single-cell gel electrophoresis assay (comet assay) and gene expression analysis were used to provide information as to the mode of action for the mutation induction. 15,178/97F mouse lymphoma cells were treated with different doses of 5-nm silver particles up to 6 ng/μl. The treatment resulted in a dose-dependent mutation induction. The mean mutation frequency for treatment with 5 ng/μl nanosilver was about 7-fold higher than the untreated control (426 x 10^-6 vs. 60 x 10^-6). The comet assay was performed with a 4 hour-treatment using similar concentrations as those for the gene mutation assay. No induction of DNA damage was found for the nanosilver treatment in the normal comet assay. Oxidative DNA damage, however, was induced by the treatment in a dose-response manner when formamidopyrimidine DNA glycosylase (FPG) was introduced in the assay. Gene expression analysis using oxidative stress and antioxidant defense PCR array (SABiosciences) showed that expression of 15 out of the 84 genes on the arrays were altered in the cells treated with the nanosilver in a concentration of 5 ng/μl. These genes are involved in production of reactive oxygen species, oxidative stress response, antioxidants and oxygen transporters. The results suggest that the 5-nm silver particles are mutagenic in mouse lymphoma cells, and the molecular mechanisms for the mutation induction may result from the oxidative stress produced by the nanosilver.

### 1705 ANALYSIS OF PERTURBATIONS IN HEPG2 CELL CYCLE AFTER GOLD NANOPARTICLE EXPOSURES: CELLULAR RESPONSE IS DEPENDENT ON PARTICLE SIZE AND SURFACE CHARGE.

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Gold nanoparticles (AuNPs) receive much attention for their role in nanomedicine. Variation in nanoparticle size is believed to yield different biomedical applications including in situ biological fluid sensing, applications in imaging, and eventually therapeutics. However, the effect on cell cycle after exposure to gold nanoparticles has not been systematically studied. Only a few studies have reported mechanistic findings on G1-phase arrest in AuNP-treated samples. Here, we present the intracellular uptake of different sizes and surface-coatings of AuNPs. The cytotoxicity, cell cycle, and DNA damage in human hepatocellular carcinoma (HepG2) cells were probed. We have synthesized AuNPs of different sizes, 10nm and 30nm, and coated them with glycine or streptavidin. The observed size distributions vary with time. Analysis of the cell cycle protein p21 and p27. CyclinD1, Retinoblastoma protein (Rb) using western blots showed that the expression of the protein levels depend on the size, surface charge, and coating of the AuNP in buffered media. We have also observed differential expression of caspases protein. Furthermore, the observed perturbations in cell cycle after exposure to these gold nanoparticles change over time.

### 1706 THE SURFACE FUNCTIONALITY OF GOLD NANOPARTICLES IMPACTS EMBRYONIC GENE EXPRESSION RESPONSES.

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In our previous studies, gold nanoparticles (AuNPs) that were cationic-functionalized, N,N,N trimethylammoniummethanethiol (TMAT), and anionic-functionalized, 2-mercaptoethanesulfonate (MES), with 0.8 and 1.5nm core size produced differential developmental responses in a sensitive embryonic zebrafish assay. The most susceptible developmental stage to nanomaterial exposure is 12-24 hours post fertilization (hpf) and 24-48 hpf for TMAT and MES AuNPs, respectively. To measure if inappropriate cellular death was occurring, embryos were exposed to the six AuNPs at 24 and 48 hpf and stained with acridine orange. The cellular death response was size dependent as exposure to 0.8nm AuNPs induced an increase in cellular death, while exposure to 1.5nm AuNP repressed overall cellular. To investigate the molecular mechanisms underlying these toxic responses, a global gene expression study was conducted with NimbleGen zebrafish microarrays using RNA isolated from embryonic zebrafish exposed to the 100% effective concentrations (EC100s) for TMAT AuNPs (50 ppm) and MES AuNPs (10 ppm) at 24 and 48 hpf. While the cores of these AuNPs are identical, the presence of the different surface functionality produced unique gene expression changes. At 24 hpf, TMAT AuNPs led to the differential expression of 106 transcripts, while MES AuNPs only impacted the expression of 8 transcripts. At 48 hpf, there were 88 differentially regulated transcripts in the TMAT exposed group, while AuNPs were 248 differentially expressed transcripts in the embryos exposed to the MES AuNPs. A more thorough investigation of the role of these differentially regulated transcripts in producing toxicity is underway. This research is supported by NIEHS P3000210, ES016896, EPA RD-833320, and Air Force Research Laboratory #FA6922-05-1-5041.

### 1707 DIFFERENTIAL SEQUENCING FOR THE CHARACTERIZATION OF SURFACE MODIFIED GOLD NANOPARTICLES IN WHOLE BLOOD.

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One of the major challenges in the evaluation of nanomaterial toxicity is the characterization of nanomaterials, particularly during the course of an exposure. Measurements of particle characteristics during exposure can yield critical information about particle properties and interactions; however, these measurements tend to be the most difficult to obtain. Conventional techniques are frequently invasive and/or require extensive sample manipulation, potentially introducing significant artifacts into the measurement. In this study, the intra-vascular behavior of various gold particles in a mouse model was investigated using differential sequencing,
ICP-MS and electron microscopy both in vivo and in vitro. We have examined the use of differential sedimentation rate analysis using a disc centrifuge to ascertain the size and distribution of gold nanoparticles in vitro in whole blood, and in vivo in a mouse model. In contrast to some other particle analytical techniques, sedimentation analysis is not as easily confounded by the complex mixtures present in whole blood. Differences in settling rate allow for high resolution analysis of multicomponent systems. The results provide insight into the aggregation behavior and distribution of parentally administered gold nanoparticles with native and PEG modified surfaces.

1708 TRANSCRIPTIONAL PROFILING OF LIVER RNA FROM MALE RATS TREATED WITH A BRISTOL-MYERS SQUIBB COMPOUND MODULATING GABA ACTIVITY.


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In an oral exploratory study in rats, treatment with 150 mg/kg/day Bristol-Myers Squibb Compound X (GABA modulator) resulted in CNS-related clinical signs including ataxia, decreased activity, recumbency, low body posture, and abnormal licking/chewing/mouth movements. These signs generally accommodated with repeated dosing. Mean absolute liver weights were increased by 30% compared to controls and these animals exhibited a slight elevation of serum ALT and AST levels. RNA toxicogenomics analyses were conducted to assess transcriptional change in the livers of drug-treated animals. Toxicogenomics analyses of the 150 mg/kg/day male livers revealed a modest level of total transcriptional change (3% of transcripts significantly altered, p<0.01) related to drug treatment. Principle component analysis (PCA) showed that treated male livers clustered separately from control livers in PCA space. In addition, genes regulated through steroid hormone receptors including AHR, PXR, and CAR had significantly altered expression. Genes specifically associated with the metabolism and clearance of thyroid hormones were induced. Ingenuity Pathway Analysis (IPA) also predicted changes in xenobiotic metabolism associated with steroid receptor activation (AHR, PXR, and CAR). Other pathways affected included oxidative stress and fatty acid metabolism. Potential toxicological outcomes predicted by pathway analysis included liver, renal, and cardiac effects. Overall, the hepatic transcriptional changes suggest a mechanism for increased liver weights and predicted thyroid hormone effects which were seen with longer term dosing.

1709 MODULATION OF GENE EXPRESSION BY GOLD NANOPARTICLES OF DIFFERENT SIZE AND SHAPE.

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Nanostructures have many applications from consumer products to therapeutic and diagnostic tools. In spite of what has been achieved so far, understanding how cells interact with nanostructures at the molecular level remains poorly understood. However, it has been suggested that the extent of biological interaction of nanoparticles (NP) is largely size-dependent. In order to better understand the nature of the biological interaction of NP, we aimed to look at the in vitro cytotoxicity, cellular uptake and gene expression of gold NP of three sizes (10nm, 50nm, 60nm) and two shapes (sphere and rod) in cultured human primary hepatocytes. Mitochondrial function assays were conducted to measure the cytotoxic effect of NP on cultured cells. Studies of cells dosed with spherical NP in concentrations ranging from 1-10 μg/mL showed no significant decrease in cell proliferation in the 10, 30 or 60nm sizes when compared to controls. However, PEG coated nanorods showed a dose-dependent decrease in mitochondrial function (<5μg/mL). TEM images revealed the localization of the Au NPs inside membrane-bound structures approximately 500nm in size, suggesting endosomal containment. To study the effect of gold NP on human gene expression, human keratinocytes were incubated in sub-toxic concentrations of NP for 6 or 24 hours. After exposure to NP, total RNA was isolated and a biotinylated complementary RNA (rRNA) was synthesized according to Affymetrix protocol for expression analysis. GeneSpring and Ingenuity Pathway analysis software showed that multiple genes from various key metabolic pathways were changed after treatment with 10nm 30nm and 60nm particles and nanorods. In summary, non-toxic levels of Au NPs were internalized by the cell and modulated gene expression. These results provide a better understanding of the molecular interactions of NP and may ultimately lead to development of novel nanodevices, diagnostics and therapeutics.

1710 THE ANTI DIABETIC DRUG METFORMIN INHIBITS PMA-INDUCED MATRIX METALLOPROTEINASE-9 ACTIVATION THROUGH A CALCIUM AND PKCα/ERK/AP-1-DEPENDENT PATHWAY.

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The aim of our study was to determine the effect of metformin on tumor invasion and migration and the possible mechanisms involved in this inhibition were investigated in human fibrosarcoma HT-1080 cells. Metformin suppressed PMA-enhanced expression of MMP-9 protein, mRNA and transcription activity levels through suppression of AP-1 activation. In addition, metformin strongly repressed the PMA-induced phosphorylation of ERK, JNK and PKCα whereas the phosphorylation of p38 MAPK was not affected by metformin. Metformin is known to activate AMP-activated protein kinase (AMPK). However, metformin-mediated decrease in MMP-9 activation was not reversed by co-treatment with AMPK inhibitor. PMA-treatment of HT-1080 cells led to a free intracellular calcium, MMPs activity and migration. Metformin decreased the PMA-induced calcium influx. Furthermore, treatment with an intracellular calcium chelator (BAPTA-AM) or a selective calmodulin antagonist (W7) caused a marked decrease in PMA-induced MMP-9 secretion and cell migration, as well as ERK and JNK/AP-1 activation. In conclusion, we demonstrated that the antitumor effects of the metformin on the PMA-induced HT-1080 cells might be through inhibiting the calcium influx, phosphorylation of PKCα, ERK, JNK and reducing AP-1 activation, leading to downregulation of MMP-9 expression.

1711 METALLOTHIONEIN ENHANCES VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION THROUGH THE ACTIVATION OF HIF-1α.

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Metallothionein-I, II (MT-I, II) are metal-binding proteins that are expressed in many tissues and protect cells and organs against metal toxicity and oxidants. MT-I and MT-II contribute to the resistance of mammalian cells to reactive oxygen intermediates and that may regulate both cellular proliferation and apoptotic pathway. VEGF, a hypoxia-inducible endothelial cell mitogen, has been characterized as a potent vascular permeability a critical factor in vasculogenesis and angiogenesis. In this study, we investigated the effects of hypoxia-inducible factor Iαfha (HIF-1α) activation by MT-II in the human endothelial-like ECV304 cell line under normoxic conditions. Treatment with MT-II under normoxic condition increased stabilization and transactivation of HIF-1α. MT-II enhanced transcriptional activity of HIF-1α in these reporter genes encoding hypoxia response element or VEGF promoter. Furthermore, MT-II induced the phosphorylation of ERK and AKT in the regulation of HIF-1α stabilization. These results demonstrate that MT-II regulates HIF-1α activity through a PI3K and ERK dependent pathway, resulting in increased VEGF expression.

1712 A SCREEN FOR NOVEL REGULATORY AGENTS OF CYP251.


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CYP251 is characterized as an “orphan” cytochrome P450 largely due to the lack of knowledge regarding its functionality. We examined the role of nuclear receptor ligands in CYP251 regulation in several cell lines which we determined had significant CYP251 expression. Treatment of A549, ASPC1 and MDA-MB-231 cells with demethasone (dex) significantly decreased CYP251 expression within 48 hours. 9-cis retinoic acid (9cRA) and all-trans retinoic acid (ATRA) significantly upregulated CYP251 expression in A549 cells within 48 hours. Treatment with PPAR agonists did not have a significant effect on the expression of CYP251 in any the cell lines.
Potassium bromate is a chemical oxidizing agent and common disinfection byproduct of surface water ozonation. Chronic drinking water exposure to potassium bromate causes renal cell tumors in male and female rats at concentrations of 125 and 250 ppm, respectively. Increased susceptibility to renal tumors in the male rat may be related to the accumulation of alpha-2u globulin and increased cell proliferation in response to bromate concentrations of 50 ppm and higher. To better characterize the molecular events that precede bromate-induced renal carcinogenesis, we have evaluated differential gene expression in male and female rat kidney following a 28-day drinking water exposure to 125 and 400 ppm potassium bromate. Total RNA samples were isolated from rat kidney cortex from 4 individual animals per treatment group, processed to cRNA, and hybridized to Agilent microarrays using a one-color procedure. The largest number of differentially expressed genes was found to be influenced by gender followed by the 400 ppm bromate dose. Common differential gene expression observed in both male and female rats exposed to 400 ppm was also observed in male, but not female rats exposed to 125 ppm bromate. Many differentially expressed mRNA transcripts were involved in tissue injury, including KIM-1 (Haver1) and osteopontin (Sp1). 8-oxoguanine DNA-glycosylase 1 (Ogg1) was not statistically different in either male or female rats. These results demonstrate that gender specific responses in gene expression associated with tissue injury at the 125 ppm bromate dose may be predictive of increased susceptibility of the male rat to bromate-induced renal carcinogenesis. Funded by Joseph Cotruvo LLC, AwwaRF 4042, Env. Abu Dhabi, IOA, MWDSoCA, Veolia, Singapore PUB, LADWP, NWRI, SNWA, Ga Cancer Coal., UGA.

Thyroid hormones (THs) play a critical role in growth, development and metabolism primarily through transcriptional regulation of targeted genes. We are currently investigating the effects of hyper- and hypothyroidism on gene expression in juvenile mice liver to develop a stronger understanding of the mechanisms by which thyroid disrupting chemicals impair development. Hypothyroidism was induced from post natal day (PND) 13 to 15 by adding model thyroid toxicants methimazole and sodium perchlorate to drinking water of pregnant females. Hyperthyroidism was induced by intraperitoneal injections (i.p.) of THs at PND 15, 4 hours before decapitation and tissue collection. Gene expression was examined by hybridization of hepatic RNA to Agilent mouse microarrays for hyper-, hypo- and euthyroid animals. MAANOVA has identified over 400 genes that are differentially regulated with a false discovery rate-adjusted p-value less than 0.05 in at least one treatment condition. Regulation of well characterized TH-sensitive genes was observed, including up-regulation of deiodinase-1 and spot-14 in hyper-thyroid animals with concomitant down-regulation in hypothyroid animals. In addition, hundreds of novel candidate genes, potentially directly regulated by THs, are being validated using alternative assays. Primary affected pathways were involved in oxidative stress response, xenobiotic metabolism, glutathione metabolism and thyroid receptor/retinoid x receptor activation. These results provide insights into the thyroid hormone-regulated transcriptome of the juvenile mouse liver. This research is part of an ongoing project aimed at establishing a tissue specific model to assess hepatic effects of environmental chemicals.

Micro-RNAs (miRNAs) are non-coding RNAs that control a wide range of biological processes through post-transcriptional repression of their target genes. At least 30% of human genes are estimated to be regulated by miRNAs, and many biological pathways including the response to environmental chemicals are thought to be affected by miRNAs. Here we examined the involvement of miRNAs in the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced liver toxicity. C57/BL/6 male mice (9 weeks old) were intraperitoneally administered either a single oral dose of TCDD (50 μg/kg) or vehicle, and sacrificed to examine the changes in the expression level of miRNAs and liver toxicity. To determine the expression level of miRNAs and their target genes in the liver, we performed quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). As an indicator for liver toxicity, we measured plasma AST level. We found that TCDD administration downregulated the expression level of miR-101a in the liver. Time course analysis of miR-101a indicated that Mir-101a was known to downregulate the postnatal-endoperoxide synthase 2 (PTGS2), which is responsible for the prostanooid biosynthesis involved in inflammation. Increase in plasma AST level indicated hepatic inflammation on 14 days after administration. Target genes of miR-101a, including COX-2, were on the increasing tendency on 2 and 14 days after administration. We revealed that miRNA regulation is affected by environmental chemicals such as TCDD, warranting studies whether and how miRNA regulation is involved in toxicities elicited by TCDD.

Organophosphorus insecticides (OP) have been widely used for many years in both agriculture and domestic residences. As a result of their extensive use, a large population is potentially exposed to low doses of OP, mainly due to the presence of these compounds in food and drinking water. Annually, thousands of cases of acute OP poisoning have been reported around the world, being suicidal, accidental, or occupational. In order to exert their toxic effects, most of the OP requires cytochrome P450 activation via oxidative desulfuration. OP acute toxicity is caused by the oxon metabolites through the inhibition of acetylcholinesterase. Oxons and a large number of metabolites are biotransformed via Glutathione S- transferases (GST). The GST catalyzes the nucleophilic attack of the tripeptide glutathione (GSH) on electrophilic substrates, thus forming an important line of defense and protecting cell components from reactive molecules. The action of GST on OP pesticides can lead to activation or detoxification. The major goal of the present study was to test the hypothesis that OP pesticides (methyl parathion) modulate the expression of GST and GSTA genes in HepG2 cells cultures. HepG2 cells were exposed to 2μM to 10 μM of methyl parathion during 24, 48 and 72 hours. After treatment, cells were harvested and cellular contents were extracted, and mRNA, protein, and activity levels were determined. Treatment with methyl parathion resulted in an increase of GST and GSTA mRNA and protein levels compared to untreated cells cultures. Our results suggest that methyl parathion was able to modulate transcriptionally GSTT and GSTA genes. Studies are in progress to define the molecular mechanisms by which methyl parathion induce the expression of GSTT and GSTA.

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enzymatic activity (EROD), and cell health. mRNA expression was determined using quantitative nucleic acid protection assays (qNAPA™) (HTG, Tucson, AZ). Fourteen liver-relevant human genes (metabolism and transporter) were monitored (ABC1, ABCB1, ABCG2, SLC01B1, CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, UGT1A1, GSTA2, SULT2A1, HMGC2S2) based on their importance in liver and sensitivity to key receptor pathways (AhR, CAR, PXR, PPARα, FXR). Expanded data analysis of the more than 300,000 data has revealed some interesting observations. EROD enzymatic activity correlations of efficacy responses across all 309 chemicals demonstrate high correlation with mRNA induction responses with CYP1A1>CYP1A2. Gene-gene correlations across all chemical responses based on dynamic range statistics revealed notable correlations of adaptive response that included a strong correlation between ABCB1 (P-gp) and ABCG2 (BCRP) consistent with their transporter activities, but much higher than that between ABCB1 and CYP3A4 (known to be co-regulated by PXR, as the major xenobiotic response pathway). Finally, a novel observation that the PPARα and cholesterol synthesis gene HMGC2S2 was profoundly suppressed (concentration-related) by chenodeoxycholic acid (CDCA) consistent with an adaptive response to increasing bile acid content to reduce precursor cholesterol synthesis.

**1718 DIURNAL VARIATION OF CYTOCHROME P450 2S1 EXPRESSION IN PULMONARY EPITHELIAL CELLS.**

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Cytochrome P450 (CYP) enzymes are monoxygenases that are capable of metabolizing endogenous substrates such as steroids, fatty acids and retinoids. fatty acid, and thus it belongs to the CYP2 family, cytochrome P450 2S1 (CYP2S1) exhibits several features that are restricted to CYP1 family such as induction by dioxin and hypoxia. CYP2S1 enzymes are highly expressed in extra-hepatic tissues including the skin, respiratory and gastrointestinal tract. CYP2S1 is tightly regulated at both the expression and functional level. Alteration of CYP2S1 is also observed in hyper-proliferative diseases and correlates with adverse outcomes. These results suggest an important role of CYP2S1 in the metabolism of endogenous substrates. Some metabolic enzymes exhibit transcriptional regulation by changes in circadian rhythms. Nuclear hormone receptors such as REV-ERBα and RORα are important regulators of circadian rhythms in target genes containing retinoid-related orphan receptor response element (RORE). Analysis of the CYP2S1 promoter demonstrated a putative RORE within the first 500 bases of the promoter, suggesting a possible role of nuclear receptors in CYP2S1 regulation. Consistent with this role, a luciferase reporter construct containing a 2000 base pair region of the CYP2S1 promoter displayed cyclical two-fold changes in reporter activity over time. Our preliminary data supports the influence of diurnal rhythms on CYP2S1 expression in human pulmonary cells. This work is funded through an NMSU Interdepartmental Research Grant (IRG) and NMSU startup funds.

**1719 STIMULATION OF INFLAMMATION AND MITOCHONDRIAL DYSFUNCTION PATHWAYS BY ARSENIC EXPOSURE IN LIVERS OF APOE-KNOCKOUT MICE.**

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Cardiovascular disease (CVD) is the number one cause of death in America and Europe. By the time CVD is diagnosed, underlying diseases such as atherosclerosis may be in advanced stages. Arsenic is a world-wide contaminant of drinking water. Arsenic exposure is known to contribute to CVD and atherogenesis, but the mechanism of disease progression is not well understood. In this study we look at the effects of arsenic exposure on the liver as a key player in the acceleration of atherosclerosis. Apolipoprotein E-knockout (ApoE-/-) mice were given drinking water ad libitum containing 49 ppm arsenic as sodium arsenite from age 21 days through age 70 days when the mice were sacrificed. We analyzed gene expression profiles from livers of arsenic-exposed and -unexposed mice using NIA 44K whole mouse genome microarrays. Network analysis of differentially expressed genes revealed dysregulation of extensive molecular networks including mitochondrial function, lipid metabolism, oxidative phosphorylation, and inflammatory response. This study demonstrates that arsenic significantly disrupts expression levels of genes that are critical to metabolic homostasis. Additional mechanistic studies will help explain the role of these genes in the early stages of liver metabolic remodeling and the long-term effects of chronic arsenic exposure in cardiovascular disease. This work is supported by NIH grants ES011314, ES014443, and Intramural Research Program of NIA/NIH.

**1720 ORGAN-SPECIFIC ROLES OF CYP1A1 IN DETOXICATION OF ORAL BENZO[A]PYRENE.**

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Polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental toxicants that are largely the byproducts of combustion processes—such as cigarette smoke and charcoal-grilled food. The GI tract is the principal route of PAH exposure, even during cigarette smoking. The most thoroughly studied prototype of PAHs is benzo[a]pyrene (BaP), which is well known to be cytotoxic, teratogenic, genotoxic, mutagenic, and carcinogenic in various tissues and cell types. Previous studies from this lab have shown that oral BaP treated Cyp1a1(-/-) global knockout mice die within 30 days with immunosuppression, whereas wild-type mice remain healthy for 1 year on this high dose (125 mg/kg/day). Thus, CYP1A1 is more important in detoxication of oral BaP rather than metabolic activation. After 5 days of oral BaP, we found surprisingly low CYP1A1 levels in liver compared with that in small intestine. Hence, we hypothesized that efficient detoxication of oral BaP may take place in small intestine rather than liver. CYP1A1 expression was studied in wild-type, Cyp1a1(-/-) knockout, hepatocyte-specific Cyp1a1 knockout, and intestinal-epithelial cell-specific Cyp1a1 knockout mice as a function of hours and days of oral BaP. The peak of CYP1A1 (mRNA, protein) expression in liver occurs 12 to 16 hours after oral BaP administration. Hepatocyte-specific Cyp1a1 knockout mice remained healthy like wild-type mice, whereas intestinal-epithelial cell-specific Cyp1a1 knockout behaved like Cyp1a1(-/-) mice, dying with immunosuppression within 30 days of oral BaP. We conclude that small intestine CYP1A1, not liver CYP1A1, is critically important in oral BaP detoxication. These data could be relevant to human populations and suggest that ingestion of combustion products might not be nearly as dangerous as many scientists think. No one has ever found important (i.e. low or absent) genetic differences in human CYP1A1 basal or inducible expression.—Supported by NIH grants R01 ES014440 and P50 ES006096.
tion in the absence of an immune response. In these cases, we must track immune responses in order to evaluate drug effects. In addition, immunomodulatory drugs may cause immunosuppression that can lead to opportunistic infections, creating a need to monitor immune responses against those infections. In recent years, there have been significant advances in methods for tracking immune responses. In drug development, monkeys (e.g., macaques) are often used for studies when other species do not express the target or have insufficient homology to the intended human target or relevant biological system. These situations are becoming more common, increasing the need to induce and measure immune responses in monkeys. For human translation, it is desirable to develop and employ methods of immune monitoring that can be used in the clinic. Antigen-specific responses are preferred because they are more physiologically relevant than those driven by polyclonal stimulators or mitogens. However, there are significant challenges to measuring antigen-specific immune responses in monkeys, such as a lack of appropriate antigen, lack of specific reagents, and the inherently variable nature of immune responses in outbred populations. This session will focus on various methods currently being used to track immune responses in monkeys and how those measurements are being used to assess either efficacy or immunotoxic potential of test compounds. The session will include discussion of experiences from scientists in an academic setting, where the most current technologies are being developed, as well as experiences and challenges encountered by those in industry attempting to track immune responses in monkeys to support drug development.

1723 THE TOX21ST COMMUNITY AND THE FUTURE OF TOXICOLOGY TESTING.

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In early 2008, the National Institute of Environmental Health Sciences/National Toxicology Program, the NIH Chemical Genomics Center, and the U.S. EPA’s National Center for Computational Toxicology entered into a Memorandum of Understanding to collaborate on the research, development, validation, and translation of new and innovative test methods that characterize key steps in toxicity pathways. A central component is the exploration of high throughput screening assays and tests using phylogenetically lower animal species (e.g., fish, worms), as well as high-throughput whole genome analytical methods, to evaluate mechanisms of toxicity. The goals of the Tox21 Community are to investigate the use of these new tools to prioritize substances for further in-depth toxicological evaluation, identify mechanisms of action for further investigation, and develop predictive models for in vivo biological response. Success is expected to result in test methods for toxicity testing that are more mechanistically based and economically efficient; as a consequence, a reduction or replacement of animals in regulatory testing is anticipated to occur in parallel with an increased ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation. The initial focus of this collaboration has been on identifying toxicity-related pathways (and assays for those pathways), establishing a Tox21 library of ~10000 compounds, and developing the databases and bioinformatic tools needed to mine the resulting data. This session will inform the scientific community of progress in meeting the Tox21 goals, successful efforts to expand the collaboration nationally and internationally, novel assay platforms that have been integrated into the screening strategy, and how Tox21 data might be used for hazard identification and risk assessment.

1724 VALVULAR HEART LESIONS IN HAN WISTAR RATS DOSED WITH ALK5 INHIBITORS.

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Aberrant signaling by TGF-β and its type 1 (ALK5) receptor has been implicated in a number of cardiovascular diseases and is considered pathogenic in vivo. Pre-clinical investigation of TGF-β signalling via ALK5 plays a critical role in heart development but the role in the developed heart is poorly understood. In the current study, the pre-clinical toxicology of ALK5 inhibitors from two different chemistry scaffolds was explored. Ten-week-old female Han Wistar rats received test compounds by the oral route for a minimum of three days. Necropsy was performed 24h after the final dose and tissues were processed for histopathology. Both compounds from the two different chemistry series induced histopathological heart valve lesions. The lesions were characterised by inflammation, haemorrhage, activation of valvular interstitial cells and degeneration. Lesions were observed in all animals and at all doses tested and could be detected in all four heart valves. Immunohistochemical analysis of ALK5 in rat hearts revealed a focal expression in the valves, but not in the myocardium. ALK5 expression was unchanged in heart valves of control and treated animals. A physical dysplasia in the femoro-tibial joint was also observed in rats treated with ALK5 inhibitors, a finding consistent with a pharmacological effect described previously with this class of compound. These findings suggest that TGF-β signalling via ALK5 plays a critical role in maintaining heart valve integrity in rats.

1725 MECHANISTIC INVESTIGATION OF A RECEPTOR TYROSINE KINASE INHIBITOR–INDUCED MYOCARDIAL DYSFUNCTION IN RATS.


Hepatocyte growth factor (HGF) and its receptor, mesenchymal-epithelial transition factor (c-Met) or HGF-R play an important role in cell proliferation. Mutated forms of the c-Met/HGF-R are associated with oncogene activation and metastasis, making the c-Met/HGF-R signaling pathway an appealing target for anticancer drugs. PF-04254644 is a selective inhibitor of c-Met/HGF-R and has potent off-target inhibitory activity for phosphodiesterase-3 (PDE3) and several other PDE families. Rats given a single oral 500 mg/kg dose of PF-04254644 developed a minimal myocardial degeneration as early as 2 hrs and a moderate myocardial degeneration at 24 hrs. Immunohistochemistry evaluation on the myocardium of rats found Caspase-3 positive staining at 8 hrs postdose. An investigative study to identify potent cardiovascular mechanism(s) was conducted in rats given 40 or 80 mg/kg PF-04254644 or 10.5 mg/kg of a known PDE3 inhibitor, milrinone. Aldehydes RG230-2 chips were used to evaluate myocardial gene expression at 2, 6, 24, and 144 hrs postdose. Microarray analysis found that PF-04254644 and/or milrinone treatments perturbed PDE gene transcription and activated c-Amp signaling similarly. However, PF-04254644 treatment perturbed genes involved with intracellular calcium transporters and calcium homeostasis more than milrinone. Perturbation of genes involved in oxidative stress and apoptotic cell death pathway was also observed with PF-04254644 treatment. PDE inhibition was substantiated by an increased CREB signaling following PF-04254644 treatment of cells in a c-Amp response element binding protein (CREB) reporter gene assay. Collectively, the results suggested that c-Met inhibition of PDE families and other potential mechanism(s) contributed to the drug-induced cardio toxicity and the importance of gene expression profiling over time.

1726 EFFECTS OF TRICHLOROETHYLENE ON HEART DEVELOPMENT.

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Trichloroethylene (TCE; TRI; C2HCl3) an organic solvent used as an industrial degreasing agent. Due to its widespread use and volatile nature, TCE is a common environmental contaminant. Trichloroethylene exposure has been implicated in the etiology of heart defects in human populations and animal models. Recent data suggest misregulation of Ca2+ homeostasis alters myocyte function and leads to changes in embryonic blood flow. In turn, changes in cardiac flow are known to cause cardiac malformations. To investigate this hypothesis we dosed developing chick embryos in ovo with environmentally relevant doses of TCE (8 ppb and 800 ppb). We then isolated RNA from embryos at crucial time points in development for real-time PCR analysis of markers for altered blood flow. Based on this analysis, we observed effects on NOS-3 (Nitric Oxide Synthase-3) and Krüppel-Like Factor 2 (KLF2) expression relative to TCE exposure. Additionally, we assessed cardiomyocyte function by isolating chick E18 cardiomyocytes from embryos exposed to TCE in ovo. Cells were measured for rate of contraction after pulsing with extracellular Ca2+ and electrical stimulation at a frequency of 1.0 Hz. These functional data showed an effect on Ca2+ handling in cardiomyocytes exposed to TCE. To investigate an apparent hosome tropism effect in the heart where 8 ppb produced a stronger effect than 800 ppb, we isolated RNA from the developing heart and AV Canal to investigate the expression of several candidate Cyclophasic P450s (CYPs) related to TCE metabolism. We observed a significant induction of multiple CYPs in the developing heart after low dose TCE exposure. Together, these data suggest an apparent cardio-specificity of TCE as a teratogen and may reflect a requirement for normal calcium regulation of contractile function during organ development.
1727 NON-INVASIVE BLOOD PRESSURE MONITORING IN AMBULATORY BEAGLE DOGS.
J. Le Bigot1, A. Béat1, G. Froget1, J. Napoléon2, R. Forster1 and A. Simonnard1. 1 CIT, Evreux, France and 2 EMKA Technologies, Paris, France. Development of biologics for which Safety Pharmacology (SP) standard study designs are not well adapted led pharmaceutical industries to develop ways to integrate SP endpoints in toxicology studies to fulfill ICH guidelines. For this purpose, external telemetry, linked to a camera video, allows today continuous recording of ECG, respiration and behavior in ambulatory non-rodent models. One of the major physiological signals missing until now was arterial blood pressure (ABP) for which a surgical approach was required. The aim of this study was to evaluate the feasibility and efficacy of a novel technique of non invasive blood pressure (NIBP) recording by telemetry developed by EMKA Technologies. The study consisted firstly in evaluating the habituation of dogs to the recording equipment. Then, the second phase was to validate the model for evaluation of the potential of a drug to modify ABP. Then a cross-validation study was performed with concomitant recording of ABP signals with an implanted catheter (internal telemetry IntT) and with the NIBP system (external telemetry ExtT). Four beagle dogs implanted with telemetric devices were used. After habituation to the external telemetry equipment, they were treated with a positive control, Prazosin, well known to modify ABP. Decreases in systolic (IntT−24%, ExtT−16%) and mean ABP (IntT−24%, ExtT−25%) were demonstrated with both techniques. This study demonstrates that NIBP measured by external telemetry is an appropriate alternative method for recording ABP over long periods without preliminary surgery in the dogs.

1728 NANOPARTICLE INHALATION MODULATES ARTERIOLAR SYMPATHETIC CONSTRUCTION: ROLE OF NITRIC OXIDE, PROSTANOIDS, AND α-ADRENERGIC RECEPTORS.
T. L. Knuckles1, D. G. Frazer2, J. L. Cumpton3, B. T. Chen4, V. Castranova2 and T. R. Nurkiewicz1, 1 Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown, WV and 2National Institute for Occupational Safety and Health, Morgantown, WV. The widespread increase in the production and use of nanomaterials has increased the potential for nanoparticle exposure; however, little is known about the biologic effects of nanoparticle inhalation. We have previously demonstrated that inhalation of a manufactured nanomaterial, titanium dioxide (TiO2), induces systemic microvascular dysfunction. The purpose of this study was to determine if nanoparticle inhalation alters arteriolar sympathetic responsiveness. Rats were exposed to TiO2 via inhalation for a total lung burden of 10 μg. Twenty four hours following exposure, the spinotrapezius muscle was prepared for intravital microscopy and sympathetic nerve stimulation (2, 4, 8, and 16 Hz) was performed. Arteriolar constriction during stimulation was equivalent in the control and TiO2 exposed groups (max constriction -14 ±2.3 μm control, -13.7 ±2.5 μm TiO2). However, the addition of NG-monomethyl-L-arginine, a nitric oxide synthase inhibitor (100 μM), greatly increased the arteriolar constriction in controls (8 and 16 Hz) but not in TiO2 exposed rats (max change -57 ±4.8 control, -39.4 ±4.4 TiO2). The addition of methylxanthine, a cyclic nucleotide inhibitor (50 μM), had no effect on sympathetic arteriolar constriction in either group when compared to normal superfusate (max change -42.0±1.4 control, -29.8±3.9 TiO2). Photolamine, an α-adrenergic antagonist (1 μM), reduced sympathetic constriction in controls, but abolished this response in TiO2 exposed rats (max change -22±3.1 control, -9.7±2.9 TiO2). These data are consistent with previous findings that indicate TiO2 exposure reduces microvascular NO bioavailability, though the compensatory mechanism remains unclear. The enhanced sensitivity to α-adrenergic receptor blockade following TiO2 exposure suggests an augmented responsiveness to tonic sympathetic activity. Support: NIH RO1-ES015022 and RCI-ES018274 (TRN).

1729 QT CORRECTION IN BEAGLE DOGS AND GOTTINGEN MINIPIGS.
A. Jackson, O. Pohl and A. Lefranc. Harlan Laboratories Ltd., Itingen, Switzerland. The validity of QT correction was tested for a large cohort of dogs by the creation of an ANCOVA-based correction formula which was compared with three literature-based correction formulae (Bazett, Fridericia and Van de Water). Results were then extrapolated to minipigs. The impact of confounding factors on QT duration was investigated in both species. For dogs and minipigs, QT and confounding pa-rameters were analysed using t-tests, principal component and multiple regression analyses. An ANCOVA-based correction formula for the populations was defined, compared with the corrected QT values generated using the literature-based formula and validated for a dog population with identified test-item-induced QT prolongation. In dogs, “breeder” and heart rate (HR) were confounding factors on QT duration. The best correction was achieved with the study-based formula, followed on the same level by the breeder and dog correction formulae, and then by fixed formulae (Van de Water-Fridericia-Bazett). The generated results allowed us to establish customised correction formulae up to the level of a single study. In minipigs, HR was the only confounding factor and the formulae were ranked: Fridericia-Bazett-Van de Water. When appropriate numbers of subjects are available, study-specific correction was found to be the most sensitive approach to dissociate QT and HR. Otherwise, an in-house formula should be applied provided sufficient background data for (training) of the slope parameter “B” are available. In the absence of an in-house formula, a fixed rate adjustment formula from the literature is preferable: we suggested the use of Van de Water’s correction in dogs and Fridericia’s formula in minipigs.

1730 DIESEL EXHAUST PARTICLE EXPOSURE AUGMENTS ARTERIOLAR MECHANOTRANSDUCTION.
K. Porter and T. R. Nurkiewicz. CCRS, West Virginia University, Morgantown, WV. Pulmonary exposure to particulate matter (PM) is known to cause systemic cardiovascular dysfunction. While this laboratory has characterized microvascular dysfunction after PM exposure, the specific hemodynamic adjustments that follow PM exposure remain unclear. In addition, we have shown that exposure to diesel exhaust particles (DEP) alters microvascular endothelium-dependent responsiveness to ACh. The purpose of this study was to determine the effects of pulmonary exposure to DEP on the peripheral microvascular response to alterations in luminal blood flow. Rats were intratracheally instilled with DEP (NIST SRM 1650b, 10 or 100 μg/rat). The spinotrapezius muscle was prepared for intravital microscopy 24 hr after exposure. Arteriolar reactivity was assessed with the parallel occlusion technique. Briefly, using a micropipette, luminal flow in an arcade arteriole was increased by gently occluding the parent arteriole immediately downstream from the arcade arteriole origin. Center-line red cell velocity was measured to quantify the microvascular hemodynamic consequences of DEP exposure. At rest, diameter and volume flow were similar among groups (28-29 μm and 4-7 nl/s); however, WSR was significantly lower in the 100 μg DEP group (2600 ± 400 s⁻¹) compared to sham-controls (5600 ± 500 s⁻¹). In all groups, parallel occlusion increased volume flow compared to baseline measurements. Compared to controls, 100 μg DEP exposure significantly augmented arteriolar dilation (17% ± 5% vs 46% ± 14%) and wall shear rate (19% ± 7% vs 60% ± 13%) in response to increased flow. Preliminary data suggests the effect is present to a lesser degree in the 10 μg DEP exposure group, in both dilation (51% ± 5%) and WSR (55% ± 20%). These results suggest DEP-exposure responsiveness to physical changes in luminal flow compared to sham-controls, as well as impaired ability to properly adapt to such changes, and that the effect is dose-dependent. We speculate that this augmented responsiveness is consistent with an adaptive response to upstream vasomotor constriction. Support: NIH RO1-ES015022 (TRN).

1731 CARDIAC, RENAL AND HEPATIC MITOCHONDRIAL TOXICITY OF DOXORUBICIN IN A CHRONIC AND ACUTE IN VIVO MODEL.
G. C. Pereira1, S. P. Pereira1, J. A. Luminii2, C. V. Pereira1, J. Magalhães3, A. Ascenção4, A. J. Moreno5, M. S. Santos1 and P. J. Oliveira1. 1Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, 2Faculty of Sport Sciences, University of Porto, Porto, Portugal and 3Institute for Marine Research, University of Coimbra, Coimbra, Portugal. Sponsor: K. Wallace. The utility of the anti-cancer drug Doxorubicin (DOX) is limited due to a dose-dependent cardiotoxicity which can be acute or chronic in its appearance. The objective of the present work was to investigate chronic and acute DOX toxicity on mitochondrial bioenergetics of three different organs, including the heart, in order to determine the most affected organ in terms of disrupted mitochondrial bioenergetics. Wistar rats were chronically (7w, 2mg/Kg) or acutely (20mg/Kg) treated with DOX. Mitochondria from chronic-treated rats presented: lower electric potential (AP), state 3 and 4 respiration (heart), lower LAP, state 3 respiration and phospho-rylative lag phase (liver); increased phosphorylative lag phase and lower ADP/O (kidney). Mitochondria from acute-treated rats demonstrated: higher ADP-induced depolarization (heart) and higher or lower state 3 respiration in liver and kidney, respectively. Interestingly, cardiac and renal mitochondria from chronic-
treated rats had lower calcium loading capacity. Interestingly, in the acute study, preliminary results indicate that heart and liver mitochondria accumulate more calcium. Measurements of mitochondrial hydrogen peroxide production show that heart and liver mitochondria from chronic DOX-treated rats produce more in the presence of complex I substrates, being that effect much more evident in the heart. In contrast, heart and liver mitochondria from acute-treated rats produce less peroxides. The present work demonstrates that both chronic and acute DOX treatment results into mitochondrial alterations, which are more evident in the chronic model and in the heart, which may contribute to DOX-induced cardiomyopathy. Acknowledgments: The present work is supported by the Portuguese FCT (SFRH/BD/36938/2007 to GP, PTDC-SAU-OSM-64084-2006 and PTDC/SAU-OSM/104731/2008 to PO).

1732 INCREASED CARDIAC RISK IN CONCOMITANT METHADONE AND DIAZEPAM TREATMENT: PHARMACODYNAMIC INTERACTIONS IN CARDIAC ION CHANNELS.

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Methadone, a synthetic opioid used in the treatment of chronic pain and in maintenance of withdrawal from opioid dependence, has been linked to QT prolongation, potentially fatal torsades de pointes, and sudden cardiac death. Concomitant use of benzodiazepines, such as diazepam, in methadone maintenance treatment appears to increase the risk of sudden death. Our objective was to determine the effects of methadone and diazepam singly and in combination on the major cardiac ion channels, responsible for the cardiac repolarization, stably expressed in mammalian cells. Using automated patch-clamp technique (PatchClamp®) for ion channel current recording, we found that methadone produced concentration-dependent block of hERG (IC50 = 1.7 μM), hNav1.5 (11.2 μM tonic block; 5.5 μM phasic block), hCav1.2 (26.7 μM tonic; 7.7 μM phasic) and hKvLQT1/hminK (53.3 μM). Diazepam demonstrated much less potent block to block of these ion channels: the IC50 values were 53.1 >100 tonic and 47.7 tonic, 89.0 tonic and 82.1 phasic, and 86.4 μM for hERG, hNav1.5, hCav1.2 and hKvLQT1/hminK, respectively. Co-administration of 1 μM diazepam with methadone had no significant effects on methadone-induced block of hERG, hCav1.2 and hKvLQT1/hminK channels, but caused a 4-fold attenuation of hNav1.5 block (44.2 μM tonic and 26.6 μM phasic). Thus, although diazepam alone does not prolong the QT interval, the relief of the methadone-induced Na+ channel block may leave hERG K+ channel block uncompensated, thereby creating a potentially greater cardiac risk.

1733 RECOVERY OF CYTOCHROME C OXIDASE ACTIVITY IS REQUIRED FOR COPPER SUPPLEMENTATION-INDUCED REGRESSION OF HYPERTROPHIC CARDIOMYOPATHY IN MICE.

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Previous studies have shown that pressure overload caused decreases in cardiac copper (Cu) concentration and cytochrome c oxidase (CCO) activity, along with hypertrophic cardiomyopathy. Dietary supplementation with physiologically relevant levels of Cu restored CCO activity and reversed hypertrophic cardiomyopathy. The present study was undertaken to test the hypothesis that recovery of CCO activity is crucial for Cu supplementation-induced regression of cardiomyopathy. A cardiac-specific COX10 conditional knockout mouse model was used, which was generated by crossing a LoxP-tagged COX10 mouse with a transgenic mice expressing a tamoxifen-inducible Cre recombinase under the control of cardiac α-myosin heavy chain promoter. COX10 deletion leads to suppression of CCO activity independent of Cu status. Mice were fed an AIN-93 diet containing adequate Cu (6 ppm) and subjected to transverse aorta constriction (TAC) or sham surgery. One week after the surgery, the mice were administered with 20 mg/kg tamoxifen daily for 5 consecutive days. COX10 knockout mice showed a 50-70% reduction in cardiac CCO activity after the tamoxifen treatment, but wild-type (WT) mice did not. Five weeks after the surgery, some mice were switched to a Cu supplementation diet (20 ppm Cu) and others remained on their previous diet for 4 more weeks. Mice were harvested at wk 5 or wk 9 after the surgery. TAC caused hypertrophic cardiomyopathy at wk 5 and its further progression to heart failure at wk 9. There was no significant difference in TAC-induced cardiac hypertrophy be-

tween WT and COX10 knockout mice. Dietary Cu supplementation caused a regression of hypertrophic cardiomyopathy in the WT mice, but did not affect the pathological progression in the COX10 mice. Therefore, CCO is essential in Cu-induced regression of hypertrophic cardiomyopathy. Supported in part by a NIH grant (HL-63760 to YJK).

1734 ASSOCIATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-1 WITH CGMP-DEPENDENT PROTEIN KINASE-1 IS INVOLVED IN COPPER-INDUCED REGRESSION OF HUMAN CARDIAC MYOCYTE HYPERTROPHY IN CULTURES.

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Vascular endothelial growth factor (VEGF) has been well known to stimulate cell proliferation and differentiation. We have observed that copper-mediated regression of cardiomyocyte hypertrophy was VEGF-dependent, but copper increased the ratio of VEGF receptor-1 (VEGFR-1) to receptor-2 (VEGFR-2), along with an increase in cGMP-dependent protein kinase-1 (PKG-1) activity. The present study was undertaken to test the hypothesis that VEGFR-1 is associated with PKG-1 and their association is involved in copper-mediated regression of cardiomyocyte hypertrophy. Human cardiac myocytes (HCM) in cultures were exposed to phenylephrine (PE) at a final concentration of 100 μM for 48 hours to induce cell hypertrophy. Copper sulfate at a final concentration of 5 μM was added to the hypertrophic HCM cultures for 24 hours with the concomitant presence of PE to reverse the hypertrophy. Both hypertrophic and hypertrophic-reversed HCM cells underwent immunoprecipitation using anti-VEGFR-1 antibody or anti-PKG-1 antibody. The immunoprecipitation eluates were subjected to SDS-PAGE separation and Western blotting or LC-MS/MS analysis. The association of VEGFR-1 and PKG-1, in addition to several key proteins that were associated with either VEGFR-1 or PKG-1 or both, was detected. In order to verify the relationship between VEGFR-1 and PKG-1, cultured HCM cells were treated with siRNA targeting VEGFR-1 or PKG-1. The gene silence of VEGFR-1 blocked copper-induced activation of PKG-1 and the gene silence of PKG-1 blocked VEGF-1 activation-induced regression of cardiac myocyte hypertrophy. This study thus demonstrates that the association of VEGFR-1 with PKG-1 is involved in copper regression of cardiac myocyte hypertrophy. Supported in part by a NIH grant (HL63760 to YJK).

1735 TEGASEROD: AN ASSESSMENT OF SAFETY AND EFFICACY IN ISOLATED HUMAN TISSUE.


Tegaserod (Zelnorm™) is an orally administered selective 5-HT4 agonist effective in the treatment of irritable bowel syndrome. It exerts its action via stimulation of the enteric nerves of the gut increasing peristaltic reflexes and general gut motility, thus reducing the bloating and constipation often associated with IBS. Tegaserod was withdrawn from the market early in 2007 due to concerns of ischemic cardiovascular (CV) effects in some patients. No causal relationship between tegaserod use and CV events has been demonstrated and the manufacturers maintain pre-existing CV disease was the cause of the findings. Using ethically obtained human tissue we present efficacy and safety data obtained during investigations with tegaserod and related substances. Tegaserod was shown to potentiate EFS-induced contractions in strips of isolated human stomach in a concentration-dependent manner when compared to relevant control in large organ baths. The effect observed was less than that of 5-HT as expected when comparing a partial agonist and an endogenous ligand. Potential off-target effects of tegaserod were assessed in contractility studies of isolated human coronary arteries using both large (> 500 μm) and small (< 500 μm) arteries. No effect of tegaserod was observed in large coronary arteries except at very high concentrations (100 μM) where a constriction was observed; however, this occurred at a concentration far beyond the typical maximum plasma concentration from a 6 mg dose. In contrast, tegaserod produced variable transient and sustained contractions of small coronary arteries except at very high concentrations (100 μM) where a constriction was observed; however, this occurred at a concentration far beyond the typical maximum plasma concentration from a 6 mg dose. In contrast, tegaserod produced variable transient and sustained contractions of small coronary arteries at concentrations within the relevant clinical range (1 to 10 nM). In summary, testing in isolated human tissue preparations has shown some interesting cardiovascular effects of tegaserod, which may not have been apparent in animal models in vivo. It is apparent from the continuing number of post-market product withdrawals that current regulatory safety tests in pre-clinical species are often a poor substitute for data obtained in humans; functional human tissue assays can bridge this gap between animal in vivo tests and clinical trials.
In order to meet the scientific requirements for preclinical safety assessment studies, some NCEs or Biologics require IV infusion of large volumes for long period of time. The objective of this study was to examine the potential cardiovascular effects of two Acute Volume Overload (AVO) challenges resulting from a 30-min IV infusion of 15 mL/kg (Day 1) and 30 mL/kg (Day 3) sterile, isotonic saline in (n=4) telemetered conscious, telemetered Cynomolgus monkeys. Lipin 1 is an intracellular protein that functions in the nucleus to coactivate expression of several key regulators of cardiac energy metabolism, likely contributing to the cardiac metabolic effects of LPS.

In summary, myocardial lipin 1 expression and PAP activity are decreased 24 h after LPS administration by release of 32P from 32P-labeled PA, was also decreased. Lipin 1 gene expression by 2.2-fold (p<0.05). However, cardiac-specific overexpression in transgenic mouse heart in- itiated a transcriptional coactivator that controls expression of two Acute Volume Overload (AVO) challenges resulting from a 30-min IV infusion of 15 mL/kg (Day 1) and 30 mL/kg (Day 3) sterile, isotonic saline in (n=4) telemetered conscious, telemetered Cynomolgus monkeys.

In order to meet the scientific requirements for preclinical safety assessment studies, some NCEs or Biologics require IV infusion of large volumes for long period of time. The objective of this study was to examine the potential cardiovascular effects of two Acute Volume Overload (AVO) challenges resulting from a 30-min IV infusion of 15 mL/kg (Day 1) and 30 mL/kg (Day 3) sterile, isotonic saline in (n=4) telemetered conscious, telemetered Cynomolgus monkeys. Lipin 1 is an intracellular protein that functions in the nucleus to coactivate expression of several key regulators of cardiac energy metabolism, likely contributing to the cardiac metabolic effects of LPS.

Identifying off-target cardiac ion channel blocking effects of new chemical entities (NCEs) is typically accomplished through voltage clamp analysis of non-cardiac cell lines (CHO, HEK293) transiently or stably expressing the ion channel under investigation. While such cell lines are amenable to the preferred higher throughput automated voltage clamp methodologies, they may not fully recapitulate the native environment of the ion channel. Primary cardiomyocyte cultures provide a native environment, but have met with limited success on automated voltage clamp platforms. Human induced pluripotent stem (hiPSC) cell-derived cardiomyocytes provide a human-based model in which ion channels are expressed in their native environment and also surmount many of the vagaries associated with traditional models. The suitability of hiPSC-derived cardiomyocytes and micro-electrode array (MEA) recording technology as a cardiac arrhythmogenicity assessment platform was tested by measuring changes, or lack thereof, in MEA-recorded field potential waveforms from spontaneously electrically active syncitia of hiPSC-derived cardiomyocytes before and during cardiac compound exposure. Beta-adrenergic activation by 100nM Isoproteranol produced an approximate 2-fold increase in beating frequency. Sodium channel block by 10 – 30 μM tetrodotoxin decreased spontaneous beating frequency. Calcium channel block by 10 – 30μM nifedipine shortened the spontaneous field potential duration. hERG channel block by 100nM E-4031 or KCNQ channel block by 3 – 10μM chromanol 293B prolonged the spontaneous field potential duration. These results demonstrate that a hiPSC-cell derived cardiomyocyte / MEA platform is suitable for assessing drug-induced changes in electrical activity of a relevant human-based cardiac model.

Lipin 1 is a transcriptional coactivator that controls expression of several key regulators of cardiac energy metabolism, likely contributing to the cardiac metabolic effects of LPS.
Experimental findings from these studies reveal markedly different atherogenic responses for cigarette smoke-exposed mice based on diet type. Mice fed a high-fat diet and exposed to cigarette smoke exhibited dose-dependent increases (with maximal responses) for atherosclerotic plaque incidence, high-grade plaque development, and (plaque) lesion volume within the thoracic aorta. In terms of demonstrating a range of responses suitable for comparative toxicological testing, lesion volumes for sham- and cigarette smoke-exposed (0.16, 0.32 and 0.48 mg WTPM/L) mice were determined to be 9.37±1.30, 71.14±17.19, 156.33±14.95 and 156.33±30.55 (x 10-3 mm3), respectively. Additional cigarette smoke-mediated effects included increased high-grade plaque development and lesion volume for the aortic arch and abdominal aorta. No meaningful increases for atherosclerotic plaque incidence, grade, or volume were observed for cigarette smoke-exposed mice fed a normal chow diet. Collectively, these data demonstrate the reproducibility of dose-dependent increases for cigarette smoke-mediated atherosclerotic progression within a preclinical rodent inhalation model.

**1744 EFFECTS OF MODULATING IN VIVO NITRIC OXIDE PRODUCTION ON THE INCIDENCE AND SEVERITY OF PHOSPHODIESTERASE 4 (PDE4) INHIBITOR CI-1044 INDUCED VASCULAR INJURY.**

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Drug Induced Vascular Injury (DIVI), is an adverse finding during preclinical safety studies of many classes of drugs including PDE4 inhibitors. The mechanism(s) of injury are poorly understood. Nitric oxide (NO) produces vasodilatation and is a mediator of inflammatory signal transmission. To determine if NO has a direct role in DIVI, rats were treated with CI-1044 alone or in combination with nitric oxide synthase inhibitors L-NAME or L-NIL or nitric oxide donors SIN-1 or NOC-18. Mesenteries were collected and processed for microscopic evaluation; blood was also collected and analyzed for serum nitrite (SN), an indirect quantitative measure of in vivo NO production. Compared to the vehicle control, CI-1044 resulted in a 1.4 fold increase in SN, which was reversible by co-treatment with L-NIL. SN values for animals receiving L-NAME were reduced 1.8 fold from the vehicle control, while SN for animals receiving SIN-1 were increased 1.8 fold from control values. Co-administration of CI-1044 and L-NAME resulted in a 2.8 fold reduction in SN compared to CI-1044 alone. Co-administration of CI-1044 and L-NIL resulted in a decrease in SN compared to CI-1044 alone.
1745 INVOLVEMENT OF SHEAR STRESS IN FENOLDOPAM AND DOPAMINE INDUCED MESENTERIC MEDIAL ARTERIAL NECROSIS.


Extrapolation and relevance of drug-induced rodent vascular injury to humans has hampered drug development due to lack of understanding of the pathologic mechanisms involved. Although vasodilatation & increased shear stress (SS) have been hypothesized to be involved in the pathogenesis of these lesions, the exact role of SS on primary target cells, smooth muscle cells (SMC) and endothelial cells (EC) in vivo remain unclear. Dopaminergic DA1 agonists such as Fenoldopam and Dopamine reproducibly induce mesenteric medial arterial necrosis (MAN) in rats following single vasotocic doses. To investigate the involvement of SS in the development of MAN, rats were given vehicle, Dopamine or Fenoldopam for 4 days followed by necrospy 24 hours later. Yohimbine, an α2 adrenoceptor antagonist, also vasosactive but lacking MAN histologic lesions, was given to rats for 4 days for comparison. To evaluate the timecourse of lesion development, rats were also given vehicle or a single vasotocic dose of Fenoldopam and necropsied 1-, 4-, 6-, 12-, or 24 hours postdose. Mesentery from each rat was collected and frozen in OCT, then EC and SMC were microdissected from sections of mesenteric arteries of each rat, and RNA was amplified and analyzed. Regulation of 37 shear stress responsive genes were evaluated using TaqMan gene expression profiling. Many of the genes evaluated were confirmed to be differentially expressed by Dopamine and Fenoldopam in EC- and/or SMC-enriched samples as compared to controls or Yohimbine following 4 daily doses. A number of SS responsive genes were also shown to be differentially regulated beginning 1- and/or 4-hours post-Fenoldopam treatment and prior to histological evidence of MAN (which was initially observed beginning at 12-hours). Evaluation of this panel of genes has provided evidence of the involvement and regulation of SS responsive genes in both EC and SMC during the development of Dopamine and Fenoldopam-induced vascular injury.

1746 IDENTIFICATION AND ANALYSIS OF CIRCULATING ENDOTHELIAL MICROPARTICLES AS A POTENTIAL BIOMARKER OF DRUG-INDUCED VASCULAR INJURY.


The finding of acute drug-induced vascular injury (DIVI) in preclinical toxicity studies often leads to delays in, or termination of, drug development projects because the relevance of this finding to humans is unclear. Furthermore, there are no sensitive and specific DIVI biomarkers that readily translate into a clinic setting. Endothelial microparticles (EMPs) are small vesicles that are shed from endothelial cells into circulating blood and are found at elevated levels in a number of human diseases associated with vascular or endothelial dysfunction. The purpose of this study was to identify and quantify by flow cytometry circulating EMPs in plasma of rats treated with phosphodiesterase 4 (PDE4) inhibitor CI-1044. In this time-course study, platelet-poor plasma (PPP) was collected from male Sprague Dawley rats (5/week point), 4, 8, 16, and 24 hours following a single dose, 24 hours following either 2 or 3 daily doses, and 10 days following 3 daily doses of 80 mg/kg/day CI-1044. Detection of EMPs in PPP was accomplished using markers specific for endothelial cells (CD31, CD106, CD146, CD54, Flk-1, VWF). Additional exclusion markers (CD45 and CD42d) and the apoptotic marker Annexin V were included to further characterize the origin of microparticles. All stained PPP samples were processed concurrently with microbeads to set a baseline for size and EMPs were identified based on size and labeling with specific markers. Administration of CI-1044 to rats resulted in increased plasma levels of EMPs at every timepoint analyzed except recovery animals. EMPs with phenotypes of CD45-CD42d-CD31+CD54-, CD45-CD42d-CD31+CD146+, CD45-CD42d-CD54+CD146+ and CD45-CD42d-CD106+Flk-1+ were increased significantly 24 hours after drug treatment and correlated with histopathology data. EMP levels dropped to baseline levels after 10 days of recovery. The present study suggests that EMPs potentially represent a novel biomarker for DIVI observed in preclinical species.

1747 A NOVEL MECHANISM FOR ALCOHOLIC CARDIOMYOPATHY: SUPEROXIDE GENERATION IS A PIVOTAL MEDIATOR FOR SUPPRESSION OF GPDH THAT TRIGGERS CARDIAC IMBALANCE OF ENERGY UTILIZATION, OXIDATIVE STRESS, AND REMODELING.

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We tried to define whether ethanol may induce NADPH oxidase-dependent superoxide that inhibits GPDH, thereby diverting upstream metabolites from glycolysis into pathways of glucose overutilization, resulting in an imbalance of glucose and fatty acid utilization, cardiac oxidative stress and structural remodeling. Male C57BL6 mice were pair-fed an alcohol or isocaloric maltose dextrin liquid diet for two months. Chronic alcohol feeding increased NADPH oxidase expression and a decrease in GPDH expression, and also caused a cardiac glucose overutilization, shown by increased GSK-3β phosphorylation and hexokinase II expression, and fatty acid metabolism disorder, evidenced by increased PPARα (a positive mediator for fatty acid uptake and utilization) and decreased PGC-1α (an essential regulator for the optimal cardiac mitochondrial fatty acid oxidation). The imbalance energy utilization was associated with significant increases in cardiac oxidative damage, shown by 3-nitrotyrosine accumulation, cell death and neutrophil infiltration, and cardiac remodeling, mirrored by cardiac hypertrophy and fibrosis (TGF-β1 and CTGF expression). To define the critical role of superoxide, cardiac H9/2 cells were treated with 2.5% (v/v) ethanol for 120 hours, which induced significant oxidative damage, cell death, and fibrosis, as observed in the hearts of alcoholic mice. However, pre-incubation of cells with superoxide dismutase mimetic significantly attenuated ethanol-decreased GPDH expression, along with improvement of the above described pathogenetic changes. Therefore, alcohol activation of NADPH oxidase-related superoxide generation plays a critical role in depressing GPDH expression. The decreased expression and function of GPDH may be the key mediator leading to cardiac imbalance of glucose and fatty acid utilization, oxidative damage, inflammation and remodeling that eventually results in alcoholic cardiomyopathy.

1748 POLYCHLORINATED BIPHENYL INDUCED VCAM-1 EXPRESSION IS ABOLISHED IN AORTIC ENDOTHELIAL CELLS ISOLATED FROM CAVEOLIN-1 DEFICIENT MICE.

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Environmental chemical contaminants, such as polychlorinated biphenyls (PCBs) are known to be atherogenic in human and the underlying mechanism is becoming elucidated. Vascular cell adhesion molecule-1 (VCAM-1) is a critical mediator for adhesion and uptake of monocytes in the initial stage of atherosclerosis development in endothelium. PCBs may be proatherogenic by causing upregulation of VCAM-1. Caveolae are particularly abundant in endothelial cell membrane and are involved in trafficking and signal transduction. The objective of this study was to investigate the role of caveolae in PCB-induced endothelial cell dysfunction. Primary mouse aortic endothelial cells (MAECs) isolated from caveolin-1 deficient mice and background C57Bl/6 mice were treated with coplanar PCBs, such as PCB77 and PCB126. In addition, siRNA gene silencing technique was used to knockdown caveolin-1 in porcine vascular endothelial cells. Expression of VCAM-1 was observed as a proatherogenic marker in cells. Both coplanar PCBs increased mRNA and protein levels of VCAM-1 and number of adhered monocytes in porcine endothelial cells. In MAECs, VCAM-1 mRNA and protein levels were increased after exposure to both coplanar PCBs in cells with caveolin-1, whereas not significantly altered in cells absent caveolin-1. Furthermore, number of adhered monocyte was significantly increased by PCB exposure only in caveolin-1 containing MAECs. Similarly, caveolin-1 silencing using siRNA technique in porcine en-
Doxorubicin (DOX) is an effective cancer chemotherapy agent, but causes dose-limiting cardiotoxicity, due in part to generation of the reactive electrophile 4-Hydroxy-2-nonenal (HNE). Glutathione (GSH) forms conjugates with HNE (GS-HNE), an MRP1 substrate. However, GS-HNE retains toxicity, and its accumulation can inhibit Glutathione-S-transferase and cause cellular toxicity. In retrospective studies, DOX-induced acute cardiotoxicity is strongly associated with the MRP1 SNP G2012T (Gly671Val, G671V) in MRP1 protein (J Hum Genet 2001; 46:656-63). We characterized the response of HEK cells expressing G671V and H433S were 2.6-fold and 3-fold more resistant to DOX than HEKG671V, reflecting an MRP1 SNP not associated with DOX-induced cardiotoxicity, Arg433Ser (R433S). Dose responses suggest that intracellular DOX concentration was poorly correlated to DOX-induced cytotoxicity. We therefore characterized the ATP-dependent transport of GS-HNE in plasma membrane vesicles prepared from HEKMRP1, HEKG671V and HER433S5 cells. The Vmax (pmol/min/mg) for GS-HNE transport was lowest for G671V (1.152 ± 0.60) and highest for R433S (16.201 ± 5.544) compared to wild-type MRP1 (7.040 ± 0.3), while Km values (μM) were 2.8 ± 0.4, 6.4 ± 2.3 and 1.4 ± 0.3, respectively. These data suggest that cells expressing the G671V SNP are more sensitive to DOX-induced cytotoxicity than cells expressing wild-type MRP1 due to their decreased ability to eflux GS-HNE. These data may explain the clinical phenomenon that patients who have the G671V SNP are prone to doxorubicin-induced acute cardiac toxicity. (CA139844)
Non-human Purkinje fiber (PF) action potential (AP) assays are commonly used to assess cardiac risk of drugs in preclinical development, but the recent availability of human stem cell-derived cardiomyocytes (SC-CMs) offers potential advantages. The electrophysiological and pharmacological profiles of human SC-CMs were compared to rabbit and canine PFs. The effects of reference compounds were measured in ventricular-type SC-CMs by perforated-patch, current clamp recording and compared with results obtained in rabbit AP assays. AP prolongation in SC-CM was observed upon exposure to hERG channel blockers (terfenadine, quinidine, cisapride, sotalol, E-4031 and verapamil), with shorter latencies than in PF assays. For torsadogenic compounds terfenadine and quinidine, SC-CM sensitivity to AP prolongation was greater than that observed in either the canine or rabbit PF assays. Moreover, the IC50 blocker chromanol 293B prolonged APs from SC-CMs, whereas both rabbit and canine PF assays are known to be insensitive to IC50 blockers in the absence of adrenergic preconditioning. In conclusion, SC-CMs provide an attractive alternative to PF AP assays; the SC-CM assay is conducted in human myocytes, removes diffusion delays with consequent reduced test compound consumption, and demonstrates an overall pharmacological sensitivity that is greater than conventional rabbit or canine PF assays.

**VALIDATION OF AN INSTANT HEK 293 CELL LINE FOR REGULATORY CARDIAC SAFETY TESTING.**


In accordance with ICH S7B regulations, all novel therapies must be investigated for interaction with the hERG potassium channel. Generally this is performed in cultured cells which are passaged in the traditional way with testing performed at a prescribed confluence. Herein, we present data obtained using “instant” HEK 293 hERG cells (CytoMetics AG), which are not cultured prior to use. On each experimental day, a cap of cells was removed from liquid nitrogen, thawed, resuspended in extracellular solution and placed in the CytoMetics Cell Reservoir. This reservoir periodically agitated the cells maintaining the suspension and preventing aggregation thereby creating optimum conditions for the Instant Cells throughout the experimental day. When required, a small aliquot of cells was transferred from the cell reservoir to the recording chamber and, using manual patch clamp techniques, IC50 values for three known hERG inhibitors (E-4031, terfenadine and cisapride) were generated.

**ROLE OF MRNA SPECIES AND ALTERED MRNA TRANSLATION IN DOXORUBICIN AND QUINONE MEDIATED CARDIOTOXICITY IN VIVO AND IN VITRO.**

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Doxorubicin, one of the most widely used and effective anticancer drugs, is limited in therapeutic use by cardiotoxicity. Here we investigated the role of the miRNA species and mRNA translation in the mechanism of doxorubicin toxicity. Cardiac damage was characterised at selected dose levels (acute 15mg/kg, chronic 2mg/kg/week for 7 weeks). Translation of mRNA was measured by performing a density gradient separation of mRNA prior to hybridisation on a whole genome microarray. miRNA (Sanger mirbase 8.1) levels were measured using microarrays (n=5). Pathway analysis implicated perturbation of electron transport chain (ETC) function as the primary mechanism for doxorubicin toxicity. mtDNA copy number was significantly increased which correlated with decreased ATP production and activation of AMPK. Differential mRNA translation was confirmed by secondary analysis. To test the hypothesis that the cardiotoxicity of doxorubicin could be mediated by inhibition of DNA replication and inhibition another redox active quinone 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) was tested in a similar manner. DMNQ is redox active but lacks the effects on DNA transcription which are the pharmacological activity of doxorubicin. The data obtained were very similar to that from doxorubicin, and implicated effects on the ETC as the main route of toxicity with alterations of mRNA translation as a response. miRNA expression changes were correlated bioinformatically with alteration of mRNA translation implicating a role for miRNAs in doxorubicin toxicity via the ETC. HL-1 cells have been utilised to test the hypotheses for specific mRNA involvement in mRNA translation, by the use of antagonism to those miRNA species of interest.

**MULTI-COMPARTMENTAL PK-PD MODELING OF BACLOFEN.**

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Pharmacokinetic and pharmacodynamic (PK-PD) modeling is a tool that allows for a predictive and descriptive relationship between time course and effect of a drug in the body. PK-PD modeling can be used to determine the potential consequences of drugs in the body. Baclofen, a GABA-A agonist, is indicated for muscle relaxation and as an antispasmodic agent. It is contraindicated in individuals who have syncope, seizures and/or a history of cardiovascular complications. This study determines the effects of baclofen on the cardiovascular and thermo-regulatory systems in the rat. Rats were housed in the novel Baxi® CUnex automated blood sampling system with integrated DS1 radioTelemetry (CUTE) system. They were dosed with baclofen (10 mg/kg i.p.) and blood pressure, heart rate and body temperature were recorded for 24 hours. Blood was also sampled in the same animals simultaneously for PK analysis. Available data was used to perform multiple PK-PD analysis using three effect compartments - BP, heart rate and body temperature. Baclofen dose dependently increased (p<0.05) BP and heart rate while decreasing (p<0.05) body temperature. PK-PD modeling indicates that baclofen differentially affects the cardiovascular system. Blood pressure responses fit into a simple Emax model where BP increases up to a maximum. Heart rate and temperature fit to a composite stimulatory and inhibitory (Emax/limax) model. Heart rate and body temperature initially decreased at low modelled effector-site concentrations then increased at higher modelled effector-site concentrations. Multi-compartment approaches to PK-PD analysis using CUTE is a powerful approach to predictive science with high statistical power. Evaluation of multiple compartments in one rat represents a way of characterizing drug effects, their outcomes and possible causal relationships between organ systems.
GINKGO BILOBA EXTRACT INDUCES GENE EXPRESSION CHANGES IN XENOBIOTICS METABOLISM AND THE MYC-CENTERED NETWORK.

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The use of herbal dietary supplements in the United States is rapidly growing and it is crucial that the quality and safety of these preparations be ensured. Ginkgo biloba has been one of the most widely sold products in health food stores in the United States, with total sales exceeding $100 millions. To date, it is still a challenge to determine the mechanisms of toxicity induced by mixtures, such as herbal dietary supplements, that contain many chemical components. We previously proposed that analyses of the gene expression profiles using microarrays in the livers of rodents treated with herbal dietary supplements is a potentially practical approach for understanding the mechanisms of toxicity. In this study, we utilized microarrays to analyze gene expression changes in the livers of male B6C3F1 mice administered Ginkgo biloba leaf extract (GBE) by gavage for two years to determine pathways and mechanisms associated with GBE treatments. Analysis of 31,802 genes revealed 129, 289, and 2011 genes significantly changed in mice treated with 300, 600, and 2,000 mg GBE/kg body weight, respectively, when compared with control animals. Drug metabolizing genes were significantly altered in response to GBE treatments. Pathway and network analyses were applied to investigate the gene relationships, functional clustering, and mechanisms involved in GBE exposure. These analyses indicate alterations in the expression of genes coding for drug metabolizing enzymes, the NRF2-mediated oxidative stress response pathway, and the Myc gene-centered network named “cell cycle, cellular movement and cancer”. These results indicate that pathway and network analysis may be used to elucidate the toxic mechanisms associated with the ingestion of Ginkgo biloba.

PROTECTIVE EFFECTS OF THE ANTHOCYANIN FROM PURPLE-FLESHED SWEET POTATO ON DIMETHYLNITROSAMINE-INDUCED FIBROSIS OF THE LIVER IN RATS.

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Liver fibrosis is a chronic disease with high mortality rate and its pathophysiology includes hepatic parenchymal cell destruction. In this study, we investigated the protective effects of the anthocyanin fraction (AF) obtained from Purple-Fleshed sweet potato on dimethylnitrosamine (DMN)-induced liver fibrosis in rats. DMN treatments for 4 weeks produced a marked liver fibrosis as assessed by serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, collagen content and histopathological changes. Pretreatment with AF prior to the administration of DMN significantly inhibited the increased serum enzymatic activities of ALT and AST, and collagen content. Histopathological evaluation of the livers also revealed that AF reduced the incidence of liver fibrosis lesions. In addition, reverse transcription-polymerase chain reaction and western blot analyses revealed that AF inhibited increases in the TGF-β1 mRNA and α-smooth muscle actin protein levels by DMN. Pretreatment with AF recovered the DMN-induced decrease body and liver weights in a dose dependent manner. These results suggested that AF plays a critical protective role in DMN-induced liver fibrosis.

CURCUMIN PREVENTS DAPSONE-INDUCED METHEMOGLOBINEMIA.


Introduction: Dapsone (4-4′-diamino diphenyl sulfone, DDS) is clinically used for the treatment of leprosy. Its use is being associated to the hematological adverse effects such as methemoglobinemia and hemolytic anemia, which are related to DDS N-hydroxylation mediated by cytochrome P450 enzyme system. Methemoglobinemia is characterized by increased quantities of hemoglobin in which the iron of heme is oxidized from its ferrous to the ferric form. Curcumin is a polyphenol derived from the herbal spice turmeric. Apart from curcumin’s potent antioxidant capacity at neutral and acidic pH, its mechanisms of action include effects on cellular enzymes such as cyclooxygenase and glutathione S-transferases. Objective: The objective of the present study was to evaluate the antioxidant capacity of curcumin in DDS-induced methemoglobinemia. Method: Male Wistar rats (200-220g), n=8 per group, were used. The research was carried out in accordance with the Society’s criteria for the care and use of animals. They were treated with 200μL of 0.1, 1.0 and 30.0mg/kg curcumin (v.o. in 20% Tween 80 solution) two hours prior to 200μL of 40mg/kg (i.p.) DDS in dimethylsulfoxide (DMSO). Blood samples were collected 2 hours after DDS administration. Methemoglobin levels were assayed in blood samples by spectrophotometry at 635nm. Results: Preliminary studies of our group evaluated control groups in which were administered 20% Tween 80 solution (v.o. 200μL), DMSO i.p. (200μL), 0.1, 1.0 and 30.0mg/kg of curcumin (v.o. 200μL) and have not induced methemoglobin compared with the blank group. Methemoglobin percentage was higher in association of 1.0mg/kg curcumin + 40mg/kg DDS (21.17%) and 30.0mg/kg curcumin + 40mg/kg DDS (26.39%). There were a significant decrease (p<0.05) on methemoglobinemia when it was associated 0.1mg/kg curcumin + 40mg/kg DDS (3.97%). Conclusion: Low concentration of curcumin associated with DDS has shown antioxidant properties and reduced methemoglobinemia. This research was supported by CAPES and FURP.

CYC450 DIETARY INHIBITORS BERGAMOTTIN INHIBITION OF TUMOR INVASION VIA SUPPRESSING PKCθ/P38 MAPK AND JNK/NF-κB-DEPENDENT MMP-9 ACTIVATION IN FIBROSARCOMA CELLS.

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The inhibitory effects of bergamottin, a CYP450 inhibitor from Citrus paradisi (grapefruit), on tumor invasion and migration and the possible mechanisms involved in this inhibition were investigated in human fibrosarcoma HT-1080 cells. Bergamottin reduced phorbol-12-myristate-13-acetate (PMA)-induced activation of MMP-9 and MMP-2 and further inhibited cell invasion and migration. Bergamottin suppressed PMA-enhanced expression of MMP-9 protein, mRNA and transcription activity levels through suppression of NF-κB activation without changing the tissue inhibitor of metalloproteinase (TIMP)-1 level. Bergamottin also reduced PMA-enhanced MMP-2 expression through suppression of membrane-type 1 MMP (MT1-MMP), but did not alter TIMP-2 levels. Bergamottin inhibited PMA-induced NF-κB nuclear translocation and 1kBα degradation, which are upstream of PMA-induced MMP-9 expression and invasion. Furthermore, bergamottin strongly repressed the PMA-induced phosphorylation of p38 MAPK and JNK, which are dependent on the PKCθ pathway. In conclusion, we demonstrated that the anti-invasive effects of bergamottin might occur through inhibition of PKCθ, p38 MAPK, and JNK phosphorylation and reduction of NF-κB activation, leading to downregulation of MMP-9 expression.

CAFFEIC ACID PHENETHYL ESTER DOWN-REGULATES 7, 12-DIMETHYLBENZANTHRENE INDUCED CYP 1A1 EXPRESSION IN HEPA-C1C7 CELLS.

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Caffeic acid phenethyl ester (CAPE), an active ingredient of propolis collected from honeybee, is known to be an antioxidant, anti-inflammatory, antiviral, and anticancer agent. However, CAPE is unknown to effects of metabolism of xenobiotics and endogenous compounds. CYP1A1 catalyzes metabolic activation of polycyclic aromatic hydrocarbons, such as 7, 12-dimethylbenanthracene (DMBA). The present study, we investigated effect of CAPE on CYP1A1 expression in mouse hepatoma Hepa1c1c7 cells. CAPE inhibited DMBA-induced DNA adducts formation in the cells. DMBA-induced EROD activity was similarly suppressed by CAPE in a dose-dependent manner. CAPE also decreased DMBA-induced CYP1A1 mRNA expression and protein level. In addition, CAPE reduced DMBA-induced XRE promoter activity. CAPE inhibited DMBA-induced aryl hydrocarbon receptor (AhR) and AhR nuclear translocation (ARNT) protein expression. Moreover, CAPE enhanced production of ROS in dose dependent manner. N-acetyl cystein, ROS inhibitor, blocked CAPE-reduced AhR protein expression. Taken together, these results suggest that CAPE has chemoprevention effect through inhibition of DMBA-induced DNA adducts formation and CYP1A1 expression mediated through AhR and production of ROS.
THE 3-CAFFEYL, 4-DIHYDROCAFFEYL QUINIC ACID FROM SALICORNIA HERBACEA PROTECTS AGAINST TERT-BUTYL HYDROPEROXIDE-INDUCED HEPATOTOXICITY THROUGH ACTIVATION OF THE NRF2-ARE PATHWAY.

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In our previous studies, we isolated a new chlorogenic acid derivative, 3-caffeoyl, 4-dihydrocaffeoyl quinic acid (CDQC), from S. herbacea and identified its antioxidant activity. Also, CDQC has been found to protect rats against carbon tetra-chloride-induced liver injury. In the present work, we investigated the protective effects of the CDQC against tert-butyl hydroperoxide (t-BHP)-induced cytotoxicity and sought to determine how CDQC protects Hepa1c1c7 cells from t-BHP-induced damage. Pretreatment of Hepa1c1c7 cells with CDQC significantly reduced t-BHP-induced generation of ROS, caspase-3 activation, and subsequent cell death. Also, CDQC up-regulated heme oxygenase-1 (HO-1) expression, which conferred cytoprotection against oxidative injury induced by t-BHP. Furthermore, CDQC induced nuclear translocation of the transcription factor NF-E2-related factor 2 (Nrf2), which is upstream of CDQC-induced HO-1 expression, and PI3K/Akt activation, a pathway that is involved in induced Nrf2 nuclear translocation. Taken together, these results suggest that the protective effects of CDQC against t-BHP-induced cytotoxicity may be due at least in part, to its ability to scavenge ROS and to regulate the antioxidant enzyme HO-1 via the PI3K/Akt-Nrf2 signaling pathways.

KAHYWEOL INHIBITS ANGIogenesis THROUGH SUPPRESSION OF STAT3 ACTIVATION IN HUMAN ENDOTHELIAL CELLS.

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Kahweol, the coffee-specific diterpene, has been reported to have anti-carcinogenic properties. Animal data support such a chemopreventive effect of coffee. However, the precise underlying protective mechanisms are poorly understood. We demonstrate that administration of kahweol inhibited MDAMB231 breast cancer and human endothelial cell migration and the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9). In addition, kahweol decreased transcription of a reporter gene under control of the VEGF promoter. Furthermore, transient expression of constitutively active STAT3 significantly reduced the inhibitory effect of kahweol on cell migration and VEGF and MMP-9 expression. Taken together, these observations indicate that kahweol inhibits angiogenesis partly through the disruption of STAT3-mediated transcription of genes, including VEGF.

SUPPRESSION OF PMA-INDUCED TUMOR CELL INVASION BY PIPERINE VIA THE INHIBITION OF PKCζ/ERK/ NF-κB AND AP-1-DEPENDENT MMP-9 EXPRESSION.

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Matrix metalloproteinase (MMP) plays an important role in the invasion and metastasis of cancer cells. Agents suppress the MMPs could inhibit the cancer cells migration and invasion. Piperine, a major component of black pepper (Piper nigrum Linn), is used as spice and nutrient enhancer. Several previous studies reported that piperine possesses various beneficial biological activities including anti-oxidant, anti-atheroslerosus, anti-inflammation, and antiarthritic properties. In this study, we examined the inhibitory effect of piperine on phosphor myristate acetaldehyde-induced MMP-9 expression in HT-1080 human fibrosarcoma cells. Piperine reduced PMA-induced activation of MMP-9 and further inhibited cell invasion and migration. Piperine suppressed PMA-enhanced expression of MMP-9 protein, mRNA and transcription activity levels through suppression of NF-κB and AP-1 activation without changing the tissue inhibitor of metalloproteinase (TIMP)-1 level. In addition, piperine suppressed PMA-induced phosphorylation of PKCζ and ERK, upstream factors involved in NF-κB and AP-1 pathways. Therefore, the inhibition of MMP-9 expression by piperine might have therapeutic potential for controlling the growth and invasiveness of cancer cells.
Matrix metalloproteinase-9 (MMP-9) plays an important role in the metastasis of cancer cells. In this study, we investigated the inhibitory effects of tumor cell metastasis by aqueous extract isolated from Prunella vulgaris (PVAE) using in vitro and in vivo assays. PVAE reduced PMA-induced activation of MMP-9 and further inhibited cell invasion and migration. PVAE inhibited PMA-induced expression of MMP-9 mRNA, protein and transcription activity levels through suppression of NF-κB activation without changing the tissue inhibitor of metalloproteinase level. PVAE inhibited PMA-induced NF-κB nuclear translocation, which are upstream of PMA-induced MMP-9 expression. Also, pretreatment with NF-κB inhibitor inhibited the PMA-induced MMP-9 expression and activity. PVAE repressed the PMA-induced phosphorylation of ERK1/2, which is upstream signaling molecules in MMP-9 expression. We confirmed that the inhibitory effect of PVAE on lung metastasis and tumor cell growth using B16-F10 melanoma cells or B16-F1 melanoma cells in C57BL/6 mice. The oral administrations of PVAE reduced the lung metastasis and tumor cell growth by B16-F10 or B16-F1 melanoma cells. These results suggested that the anti-metastatic effect of PVAE is mediated through suppression of PMA-induced phosphorylation of ERK1/2 via ERK1/2 signaling pathway as well as MMP-9 activity.

1769 INHIBITORY EFFECT OF PMA-INDUCED TUMOR CELL METASTASIS BY AQUEOUS EXTRACT ISOLATED FROM PRUNELLA VULGARIS VIA THE INHIBITION OF NF-κB-DEPENDENT MMP-9 EXPRESSION.

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In this study, we evaluated the protective effect of S-allyl cysteine (SAC), one of the organosulfur compounds of AGE, against free fatty acid-induced lipotoxicity in HepG2 cells: involvement of maintenance of AMP-activated protein kinase activation.

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In this study, we evaluated the protective effect of S-allyl cysteine (SAC), one of the organosulfur compounds of AGE, against free fatty acid-induced lipotoxicity in vitro HepG2 non-alcoholic fatty liver diseases (NAFLD) models. Pretreatment of HepG2 cells with SAC significantly reduced free fatty acid (FFA)-induced generation of ROS, caspase-3 activation, and subsequent cell death. SAC inhibits cholesterol and triglyceride (TG) synthesis in a similar manner to the AMP-activated protein kinase (AMPK) activator metformin. Significant increase in AMPK phosphorylation was observed when the cells were incubated with SAC. Activation of AMPK was also demonstrated by measuring the phosphorylation of acetyl-CoA carboxylase, a substrate of AMPK, correlated with a subsequent increase in fatty acid oxidation. The results indicated that by reducing TG and lipid accumulation, SAC could protect the liver from NAFLD through the activation of AMPK. Therefore, SAC might have the therapeutic potential for preventing or treating NAFLD.

1770 S-ALLYL CYSTEINE PREVENTS FREE FATTY ACID -INDUCED LIPOTOXICITY IN HEPG2 CELLS: INVOLVEMENT OF MAINTENANCE OF AMP-ACTIVATED PROTEIN KINASE ACTIVATION.

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Psidium guajava Linn, commonly known as guava, is one of the most important economic fruits in tropical areas. Different parts of the plant are used in traditional medicine for the treatment of various human ailments such as wounds, ulcers, bronchitis, eyeesores, and diarrhea. Guava fruits are known to be a source of antioxidant. In this study, we investigated the effects of the water extract of Psidium guajava (PGE) on IgE-mediated allergic response in rat mast RBL-2H3 cells. PGE reduced antigen-induced IL-4 and TNF-α production and expression in IgE-sensitized RBL-2H3 cells. Antigen-induced phosphorylation of mitogen-activated protein (MAP) kinases was inhibited by PGE. Taken together, the in vitro anti-allergic effect of PGE suggests possible therapeutic applications of this agent in inflammatory allergic diseases through inhibition of cytokines and multiple events of FcεRI-dependent signaling cascades in mast cells.

1771 PSIDIUM GUAJAVA EXTRACT SUPPRESSES IGE-MEDIATED ALLERGIC RESPONSE BY INHIBITING MULTIPLE STEPS OF FOR SIGNALING IN MAST CELLS.

H. Hee1, 2, J. Park1, 2 and H. Jeong1, 1Pharmacy, Chungnam National University, Daejeon, Republic of Korea and 2Pharmacy, Chosun University, Gwangju, Republic of Korea.

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1772 INHIBITORY MECHANISM OF PLEUROTUS ERYNGII EXTRACT ON IGE-MEDIATED ALLERGIC RESPONSE IN MAST CELLS.

E. Han1, 2, J. Park1, 2 and H. Jeong1, 1Pharmacy, Chungnam National University, Daejeon, Republic of Korea and 2Pharmacy, Chosun University, Gwangju, Republic of Korea.

Pleurotus eryngii (also known as king trumpet mushroom, french horn mushroom, king oyster mushroom) is an edible mushroom native to Mediterranean regions of Europe, the Middle East, and North Africa, but also grown in parts of Asia. It has the ability to produce various biologically active compounds and possesses a well-developed ligninolyl enzyme system that participates in the degradation of lignin and different aromatic compounds. In this study, we investigated the effects of the ethyl acetate extract of Pleurotus eryngii (PPE) on IgE-mediated allergic response in rat mast RBL-2H3 cells. PPE reduced the β-hexosaminidase and histamine release from anti-DNP-IgE-sensitized RBL-2H3 cells. In addition, PEE extract inhibited the DNP-IgE-induced increases in IL-4 and TNF-α production and expression in RBL-2H3 cells. Moreover, PEE suppressed DNP-IgE-induced phosphorylation of mitogen-activated protein (MAP) kinases. Taken together, the in vitro anti-allergic effects of PEE extract suggest possible therapeutic applications for this agent in allergic diseases through the inhibition of inflammatory cytokines.

1773 INHIBITORY MECHANISM OF CPY1A1 EXPRESSION BY CAPSAICIN MEDIATED ARYL HYDROCARBON RECEPTOR AND CCAAT/ENHANCER-BINDING PROTEIN IN MURINE HEPATOMA HEPA-1C1C7 CELLS.

J. Im1, 2, E. Han1, 2 and H. Jeong1, 1Pharmacy, Chungnam National University, Daejeon, Republic of Korea and 2Pharmacy, Chosun University, Gwangju, Republic of Korea.

Capsaicin (CPS), a constituent of green and red peppers, has been linked with suppression of tumorigenesis and carcinogenesis. The influence of CPS on cyclohexone P450 1A1 (CYP1A1) expression and their mechanisms remain unclear. In this study, we examined the effects of CPS on 3-methylcholanthrene (3-MC)-induced CYP1A1 in mouse hepatoma Hepa-1c1c7 cells. 3-MC-inducible CYP1A1-specific 7-ethoxyresorufin O-deethylase (EROD) activity was markedly reduced by CPS in a dose dependent manner. 3-MC-inducible CYP1A1 mRNA expression and protein levels were markedly suppressed upon treatment with 3-MC and CPS, and this is consistent with their effects on EROD activity. A transient transfection assay using xenobiotic-response element (XRE)-linked luciferase and an electrophoretic mobility shift assay revealed that CPS reduced the transformation of the aryl hydrocarbon receptor (AhR) to a form capable of specifically binding to the XRE sequence in the promoter region of the CYP1A1 gene. Treatment with capsazepine, a vanilloid receptor antagonist, did not affect the suppressive effects of CPS on 3-MC-inducible EROD activity. In addition, CPS enhanced CCAAT/enhancer binding protein (C/EBPβ) mRNA and protein level in Hepa-1c1c7 cells. These results provide evidence that CPS suppresses 3-MC induction of CYP1A1 levels and that activation of C/EBPβ by CPS contributes to suppression of 3-MC-inducible AhR-mediated CYP1A1 expression.

1774 INHIBITORY EFFECT OF 3-CAFFEOLYL-4-DICAFEOYLQUINIC ACID FROM SALICORNIA HERBACEA AGAINST PMA-INDUCED CYCLOOXYGENASE-2 EXPRESSION IN MACROPHAGES.

H. Jeong1, E. Han1, 2 and H. Kim1, 2, 1Pharmacy, Chungnam National University, Daejeon, Republic of Korea and 2Pharmacy, Chosun University, Gwangju, Republic of Korea.

Salicornia herbacea (S. herbacea) has been used as a folk medicine to treat a variety of diseases such as constipation, obesity, diabetes, and cancer. In the present study, we investigated the effects of a novel chlorogenic acid, 3-caffeoyl-4-dicaffeoylquinic acid (CDCQ), isolated from S. herbacea, on cyclooxygenase-2 (COX-2) expression in murine macrophage RAW 264.7 cells. Phorbol 12-myristate 13-acetate (PMA) induces COX-2 expression and production of prostaglandin E2 (PGE2). PMA-induced COX-2 protein and gene expression and PGE2 production were significantly inhibited by CDCQ in a dose-dependent manner. Transfection of hCOX-2,
as well as of deletion and mutation promoter constructs, revealed that the CCAAT/enhancer-binding protein (C/EBP) and activator protein-1 (AP-1) predominantly contributed to the effects of CDQ. Furthermore, CDQ significantly inhibited PMA-induced activation of the MAP kinases, JNK and p38. These findings demonstrate that CDQ effectively attenuates COX-2 production, and enhance our understanding of the anti-inflammatory properties of CDQ.

**INHIBITION OF LIPID SYNTHESIS THROUGH ACTIVATION OF AMP-ACTIVATED PROTEIN KINASE BY SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM.**

H. Park 1, 2, E. Han 1, 2, H. Kim 1, 2, Y. Hwang 1 and H. Jeong 1. Pharmacy, Chungnam National University, Daejeon, Republic of Korea and 2Pharmacy, Chosun University, Gwangju, Republic of Korea.

The present studies were performed to determine the extent to which the effects of saponins from the root of Platycodon grandiflorum (Changkil saponins: CKS) on hepatocellular lipids is mediated by AMP-activated Protein Kinase (AMPK) regulates lipid accumulation in insulin resistant states. AMPK activation by Sirtuin (Sirt1) also protects against fatty acid synthesis (FAS) induction and lipid accumulation caused by high glucose. Sirt1 acts as the upstream of AMPK signaling and hepatocellular lipid metabolism. CKS increased Sirt1 and AMPK activity in human HepG2 hepatocytes exposed to high glucose. In addition, CKS increase acetyl CoA carboxylase (ACC). AMPK downstream effectors, in human HepG2 hepatocytes exposed to high glucose. CKS inhibits lipid synthesis and total cholesterol synthesis in a similar manner to the AMPK activator, 5-aminoimidazole-4-carboxamide 1-b-ribofuranoside (AICAR). CKS substantially prevents the impairment in phosphorylation of AMPK and its downstream target, ACC, elevation in expression of FAS, and lipid accumulation in human HepG2 hepatocytes exposed to high glucose. These effects of CKS are largely abolished by inhibition of Sirt1 activity, suggesting that the stimulation of AMPK. CKS lowers hepatic lipid content and inhibition of triacylglycerol synthesis by activating AMPK, thereby mediating beneficial effects in hyperglycemia and insulin resistance. In conclusion, AMPK signaling by CKS, which Sirt1 induces, may have potential therapeutic implications for dyslipidemia and accelerated atherosclerosis in diabetes and metabolism-related disease.

**MOLECULAR MECHANISMS OF THE SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM-MEDIATED ENDOTHELIAL NITRIC-OXIDE SYNTHASE ACTIVATION.**

K. Gyun 1, 2, T. Hien 2, E. Han 1, 2 and H. Jeong 1. Pharmacy, Chungnam National University, Daejeon, Republic of Korea and 2Pharmacy, Chosun University, Gwangju, Republic of Korea.

Nitric oxide (NO) produced by endothelial nitric-oxide synthase (eNOS) represents an anti-thrombotic and anti-atherosclerotic principle in the vasculature. Previous studies have demonstrated that the saponins derived from roots of Platycodon grandiflorum (CKS) inhibits tumor necrosis factor-α-induced expression of adhesion molecules in human endothelial cells. In this study, we identified that CKS increases expression of eNOS phosphorylation and NO production in human endothelial cells. Treatment of CKS increases the phosphorylations of Akt, p38/ MAPK, AMP-activated protein kinase (AMPK) and calmodulin-dependent protein kinase II (CaM kinase II) in MCF10A cells. All LAMs decreased cell viability and concomitantly induced caspase-3/7 activity in MCF7 cells in a concentration- and time-dependent way. In contrast, no decrease in cell viability nor an increase in caspase-3/7 activity was observed in MCF10A cells up to 4 hours of incubation. However after 6 hours, a significant increase of caspase-3/7 activity was observed in MCF10A cells at 10 μM (100%) and 30 μM (250%). LMM was the most potent of the LAMs tested. Relative effect potencies for LMM to induce caspase-3/7 activity were 0.0003 (MCF10A) and 0.0008 (MCF7) compared with the positive control staurosporine after a 6-hour incubation. After a 6-day incubation with these LAMs, cell viability was concentration-dependently decreased in both cell lines and IC50 values differed only by factor 2 between both cell lines. Under these conditions, MCF-7/10A cells were more susceptible to apoptosis. The most potent compound was 7, 12-dimethylbenz(a)anthracene (DMBA).

**DIFFERENTIAL INDUCTION OF APOPTOSIS IN MALIGNANT MCF-7 AND NORMAL MCF-10A HUMAN MAMMARY EPITHELIAL CELLS BY LAMELLARINS.**

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Lamellars (LAMs) are a large family of promising anti-cancer marine compounds isolated from mollusks. The LAMs have variable biological and toxicological activities, which are modulated by variable hydro- and methoxy-substitutions and the presence or absence of a C5=C6 double bond. For the present study, three LAM congeners (LMF, LMK and LMM) were investigated for their potency to induce apoptosis in the malignant MCF-7 and non-tumorigenic MCF-10A mammary epithelial cell lines. All LAMs decreased cell viability and concomitantly induced caspase-3/7 activity in MCF7 cells in a concentration- and time-dependent way. In contrast, no decrease in cell viability nor an increase in caspase-3/7 activity was observed in MCF10A cells up to 4 hours of incubation. However after 6 hours, a significant increase of caspase-3/7 activity was observed in MCF10A cells at 10 μM (100%) and 30 μM (250%). LMM was the most potent of the LAMs tested. Relative effect potencies for LMM to induce caspase-3/7 activity were 0.0003 (MCF10A) and 0.0008 (MCF7) compared with the positive control staurosporine after a 6-hour incubation. After a 6-day incubation with these LAMs, cell viability was concentration-dependently decreased in both cell lines and IC50 values differed only by factor 2 between both cell lines. Under these conditions, MCF-7/10A cells were more susceptible to apoptosis. The most potent compound was 7, 12-dimethylbenz(a)anthracene (DMBA).
1780 ANTIANDROGENIC AND ANTIPROLIFERATIVE EFFECTS OF 3,3’-DIINDOLYL METHANE (DIM) AND RING-SUBSTITUTED ANALOGS (RING-DIMS) IN LNCAP HUMAN PROSTATE CANCER CELLS.

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Cruciferous vegetables have been found to protect against prostate cancer. Indole-3-carbinol (13C) and its dimeric product 3,3’-diindolylmethane (DIM) exhibit anti-tumor activities both in vitro and in vivo. Previous studies have shown that DIM inhibits androgen receptor (AR) nuclear translocation, leading to down-regulation of target genes. In this study, we observed structure-dependent differences for the effects of the synthetic 4,4’- and 7,7’-dihalo-DIMs on AR and prostate specific antigen (PSA) expression in LNCaP cells. We also report that DIM and ring-substituted analogs (ring-DIMs) 4,4’-dibromo-, 4,4’-dichloro-, 7,7’-dibromo- and 7,7’-dichloro-DIM, inhibit the proliferation of androgen-sensitive LNCaP prostate cancer cells at 10 and 30 μM. Western blot analysis showed that 4,4’- and 7,7’-dibromo-DIMs reduced AR protein levels, whereas 4,4’- and 7,7’-dichloro-DIMs had little effect. RT-PCR analysis clearly indicated that the 4,4’-dihalo-DIMs and 7,7’-dihalo-DIMs (24h exposure) inhibited the expression of AR at the mRNA level. The 4,4’- and 7,7’-dihalo-DIMs also significantly decreased PSA mRNA expression and cellular protein secretion levels at 10 and 30 μM. These anti-androgenic effects of the dihalo-ring-DIMs suggest they may be interesting as (or form the basis for) novel agents for the treatment of hormone-sensitive prostate cancer, either alone or in combination with other therapeutics. This work will be continued with the study of apoptotic effects of ring-DIMs in LNCaP as well as hormone-insensitive PC-3 human prostate cancer cells.

1781 ACTIVATION OF THE TUMOR SUPPRESSOR P53 IS NOT ESSENTIAL FOR GROWTH INHIBITION OF HUMAN HEPATOCELLULAR CARCINOMA CELLS BY ISOTHIOCYANATES.

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In a number of studies, Brassica-derived isothiocyanates (ITCs) decreased or inhibited cell growth in cancer cell culture as well as in animal xenograft models. The induction of programmed cell death (apoptosis) is a well-documented mechanism in the anticancer activity of ITCs. A number of cell lines have been studied for apoptosis induction by ITCs, and signal transduction pathways have been investigated. It has been shown that apoptosis induction is generally mediated via the intrinsic mitochondrial death pathway; however, the receptor death pathway may also be involved. Even cancer cells, which are normally resistant to chemotherapeutic drugs due to their high expression levels of anti-apoptotic Bcl-2 family proteins, have been rendered to cell death by ITC treatment. The studies conducted by our group showed proliferation inhibition of liver cancer cells by 4-methylthiobuty1 ITC (MTBITC), which arrested cells at the G2/M phase and depolarized mitochondria at concentrations exceeding 10 μM. Although the tumor suppressor p53 was markedly expressed in treated cells compared to control, RNAi experiments demonstrated the independency of growth inhibition from p53. This detail could be very valuable for the treatment of tumors with existing p53 mutation, e.g. 30 to 50% of hepatocellular carcinomas (HCC), one of the most common malignant tumors worldwide, are affected by this mutation. The therapeutic application of ITCs for treatment of malignant tumors is promising; however, further research has to be done to characterize the concentration-dependent ambivalent character of ITCs and their specificity of tissue targeting.

1782 MODULATION OF HUMAN LYMPHOMA VIABILITY AND PROLIFERATION BY RICE BRAN FROM GENETICALLY DIVERSE VARIETIES.

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Rice provides the majority of daily caloric intake for half of humanity and is a rich source of bioactive food components (BFC) with disease prevention properties. Emerging evidence supports that BFC in rice bran may work synergistically and in parallel to elicit protective host immunity and prevent cancer, and thus focusing on nutritional magic bullets as in dietary supplements may be problematic over promoting a general increase in whole grain consumption. Metabolomics was performed using a standard operating procedure developed by our laboratory to investigate the phytochemical diversity of rice bran isolated from 10 genetically diverse rice cultivars with distinct grain characteristics. Methanol-soluble compounds were detected by liquid chromatography-mass spectrometry and analyzed using principal components analysis. Three rice varieties with distinguishable bran global metabolite profiles (>200 metabolites) and that significantly differs in vitamin E content were assessed for their ability to effect human lymphoma viability and proliferation as measured by MTT assay and CFSE staining with flow cytometry. Relative differences across rice varieties were also examined for phytate, ferulic acid, phytosterols and salicylic acid content. A 30-50% difference in viability and proliferation index was detected across rice varieties examined. Identifying the phytochemicals and rice genetic regions in rice bran that account for differential anticancer activity can be used to inform future crop improvement strategies for disease prevention. These findings support that cultivar information should be reported when investigating BFCs as unique phytochemical contents across varieties demonstrate not only differential anticancer activity as reported herein, but may also differentially influence cellular stress responses to toxins.

1783 SUPPRESSION OF LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION BY ELLAGITANNIN.

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Ellagitannin isolated and purified from Euonymus species. Ellagitannin is known to possess anti-cancer and anti-oxidative activity. Recently, ellagitannin has been reported for its anti-inflammatory properties. In our study, ellagitannin inhibited pro-inflammatory cytokine secretion and cellular protein secretion levels at 10 and 30 μM. These anti-inflammatory effects of ellagitannin may be interesting as (or form the basis for) novel agents for the treatment of inflammatory diseases, either alone or in combination with other therapeutics. This work will be continued with the study of anti-inflammatory effects and mechanisms of action of ellagitannin in macrophages. Our results suggest that ellagitannin might be a candidate for developing anti-inflammatory and cancer chemopreventive agents.

1784 CONSUMPTION OF DOCOSEHAEROXIC ACID ATTENUATES LUPUS NEPHRITIS-RELATED GENE EXPRESSION AND DISEASE PROGRESSION IN NZBWF1 MICE.

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Systemic lupus erythematosus (SLE) is a debilitating autoimmune disease of unknown etiology, where mortality is associated with the onset of lupus nephritis. Of note, n-3 polyunsaturated fatty acids (n-3 PUFA) have shown efficacy in preventing and treating lupus nephritis in rodents and human clinical studies, however, mechanistic studies are lacking. The purpose of this study was to validate the capacity of n-3 fatty acid docosahexaenoic acid (DHA) to suppress murine lupus nephritis and profile ensuing transcriptome changes in lupus-prone mice. Four week-old female NZBWF1 mice were divided into three groups and fed daily an AIN-93G diet consisting of 1% corn oil and 6% of either DHA-enriched fish oil (DHA [n-3]), high-oleic safflower oil (SAF [n-9]) or corn oil (CRN [n-6]). Plasma and urine were collected at regular intervals, and mice terminated at 16 (pre-nephritic) or 36 weeks (nephritic) for further histological, protein, and PCR analyses. At 36 wk termination, SAF and CRN alone exhibited marked nephritis (both class IV, blind ISN/PRS grading scale) and proteinuria (75% and 63%, respectively). Also, plasma anti-dsDNA IgG, an SLE biomarker, was significantly greater in SAF (216%) and CRN (240%). For gene expression profiling, DHA suppressed numerous genes in blood, kidney, and spleen samples. However, consistent suppression of Ccl7, Cxcr3, Il-4Rα, and osteopontin (OPN) was observed. Notably, OPN is a pleiotropic cytokine linked to autoantibody formation. When OPN was assessed in 36 wk plasma
samples, OPN levels were significantly influenced by diet with DHA exhibiting 62% and 80% that of SAF and CRN, respectively. Thus, DHA suppression of OPN is a potential critical event in the downregulation of aberrant humoral autoimmunity in murine lupus nephritis.

**1785 MODULATION OF BODY FAT MASS AND LEAN WEIGHT IN DEOXYNIVALLENOL-INDUCED BODY WEIGHT REDUCTION IN THE OBESE MOUSE.**

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Our laboratory has observed that deoxynivalenol (DON) can both prevent and ameliorate weight gain in the diet-induced obese (DIO) mouse model. Here we related DON’s therapeutic effects in DIO mice to food intake, body fat mass and lean weight. B6C3F1 mice (female, 11-week-old) were divided into 3 groups and fed each diet (10 kcal%, 45 kcal% and 60 kcal%) from fat, Research Diets, Inc.) for 94 days. After inducing obesity, the mice were divided into 5 groups (n=8) and fed control diet (10 kcal%) or high-fat diets (45 kcal% or 60 kcal%) with or without DON (10 ppm) from Day 94 to 147. Body weights of the 45 kcal% + DON and 60 kcal% + DON were significantly lower than the 10 kcal% control by Day 105. As compared to the 45 and 60 kcal% groups, food intake by DON-fed groups significantly decreased at Day 98 and 101, which corresponded to the period in which DON-fed groups exhibited robust body weight decreases. As determined with MRI (EchoMRI-100ST), body fat mass and body fat percentage of the 45 and 60 kcal% with DON-fed mice gradually reduced to those of control. Lean weights of the DON-fed groups were significantly lower than those of the 45 and 60 kcal% groups after Day 122. We conclude that DON-induced reduction in body weight in the DIO mice resulted from decreased food intake, and this corresponds to decreases in both body fat mass and lean weight.

**1786 NORMAL RANGE AND FORMS OF DIETARY SELENIUM PREVENTS METHYLMERCURY TOXICITY IN LONG EVANS RATS.**

N. V. Ralston, Energy & Environmental Research Center, University of North Dakota, Grand Forks, ND. Sponsor: M. Aschner.

Methylmercury (MeHg) is a highly specific irreversible inhibitor of selenium (Se)-dependent enzymes (Se-enzymes). Over 30 genetically unique Se-enzymes are expressed in mammals, many with roles in preventing and reversing oxidative damage in the brain. The Se-enzymes employ selenocysteine (Sec) at their active sites and MeHg intoxication inhibits Se-enzymes activities in brain and neuroendocrine tissues. Supplemental Se has been known to counteract Hg toxicity since 1967, but mechanisms have only recently become clear. Since dietary Se must be reduced to inorganic forms before it can be incorporated into Sec, it was not known whether dietary Se from ocean fish would be as effective as inorganic Se provided in the diet. In the current study, 120 weanling male Long Evans rats were fed diets containing either low or high MeHg (0.5 or 50 nmol MeHg/g) with Se as inorganic Se (sodium selenite) at low, normal, or rich (0.1, 1.0, or 10 nmol Se/g) in diets prepared with torula yeast protein (as~30% of nutritionally complete AIN-93G diet). Comparison was made with torula yeast protein (as~30% of nutritionally complete AIN-93G diet).

Under the European Union’s Registration, Evaluation, Authorization, and restriction of Chemicals (REACH) regulation, the Derived No-Effect Level (DNEL) represents a level of exposure above which humans should not be exposed. Chemical-specific DNELs for worker (W-DNEL) and consumer (C-DNEL) populations are often derived as part of the chemical safety assessment. The European Chemicals Agency (ECHA) has developed guidance for the calculation of DNELs for both workers and consumers. Steps in DNEL calculation include establishing study “dose descriptor(s)” such as NOAEL, LOAEL, etc. and their modification for bioavailability, route-to-route extrapolation and exposure conditions; and, lastly, application of Assessment Factors (AFs) for intra-and inter-species differences, duration of exposure, etc. While it is preferable to use chemical-specific modifiers/factors in DNEL calculations, many are not [readily] available and, thus, ECHA provides default factors for use in these calculations. The impact of both chemical- and use-specific data (vs. defaults) is illustrated by calculating W-DNEL and C-DNEL for: (1) long-term inhalation exposure based on rat inhalation data and (2) long-term dermal exposure based on oral rat data. Significant differences in W-DNEL and C-DNEL values can be observed depending on the use of chemical-specific data (e.g. dermal and oral absorption data for route-to-route extrapolation) and consumer use data (e.g. number of hours/day of exposure). For example, if both dermal and oral % absorption of a chemical is known, the use of these data can provide a significantly different DNEL vs. the DNEL calculated using ECHA defaults. As an example of case (2), above, the DNEL for a chemical with 100% and 10% absorption via oral and dermal routes, respectively, will be 10-fold greater than that calculated using the ECHA defaults. This exercise shows the importance of using reliable, available, chemical-specific data for calculation of DNELs for use in REACH compliance.

**1789 ICCVAM RECOMMENDATIONS FOR USE OF THE LLNA FOR EVALUATING THE ALLERGIC CONTACT DERMATITIS POTENTIAL OF PESTICIDE FORMULATIONS.**

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ICCVAM has updated its 1999 validation report on the LLNA based on a recent evaluation of the usefulness and limitations of the LLNA for assessing the skin sensitizing potential of pesticide formulations. This review was initiated because the original report did not include an analysis of the LLNA for these types of substances, and there were growing regulatory concerns that the LLNA might not identify sensitizing pesticide formulations. LLNA data from 104 formulations were...
included in the evaluation, most of which are water soluble and therefore were tested in an aqueous vehicle (1% Pluronic L92). Of the pesticide formulations for which LLNA and guinea pig data were available (n=23), the LLNA classified 52% (12/23) as sensitizers, while GP tests classified only 13% (3/23) as sensitizers. All three of the pesticide formulations identified as sensitizers in the GP test were also identified as sensitizers in the LLNA; there were no instances of underprediction by the LLNA. Thus, there is a greater likelihood of obtaining a positive result in the LLNA than in a GP test. These studies also provide data for aqueous solutions that emphasize the need for careful selection of an appropriate vehicle that maintains test substance contact with the skin (e.g., 1% Pluronic L92) to achieve adequate exposure when testing such substances. Based on these data, ICCVAM agreed with an international peer review panel that the LLNA could be used for testing pesticide formulations, and any other products, unless there are unique physicochemical properties that may interfere with the ability of the LLNA to detect sensitizing substances. ICCVAM recommendations are being forwarded to Federal agencies for their consideration for future regulatory acceptance. These recommendations should expand the use of the LLNA for skin sensitization testing, thereby reducing and refining animal use for this purpose.

1790 CURRENT DRUG SCHEDULING REVIEWS REPORTED BY THE DRUG ENFORCEMENT ADMINISTRATION.


As mandated by the Controlled Substances Act (CSA), DEA collects and reviews scientific, medical and other data for substances with abuse potential to determine their appropriate control status for placement into one of five schedules. Administrative process for scheduling is currently ongoing for carisoprodol, dextromethorphan, Salvinorin A and hallucinogens such as 4-iodo-2,5-dimethoxy-3,4-methylenedioxyphenethylamine (2C-T-2), and 2,5-dimethoxy-4-iodoamphetamine for possible control under the CSA. Chemical synthesis/pharmacological studies for tramadol and propofol, decontrol of sibutramine and 6-beta-naltrexol and amendment to CFR so as to allow generic products for production of all three of these substances. DEA is currently reviewing the refined approach was applied to 20 case study chemicals; this model has since been incorporated into the 1979 Convention on Psychotropic Substances. Adapting this administrative process for scheduling is currently ongoing for zipreprod, amphetamine, mesocarb, 4-methylthioamphetamine and bromizolam.

1791 USE OF LINEAR EXTRAPOLATION OF CANCER POTENCY FOR REGULATING CHEMICALS: COLLISION OF SCIENCE AND POLICY.

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Regulatory agencies extrapolate animal cancer dose-response data from high doses used in toxicity studies to environmentally relevant concentrations to which humans may be exposed. This is needed in order to develop concentrations considered acceptable in the environment, typically based on a target cancer risk of 1 in 1,000,000. The linearized multi-stage model is almost exclusively used for this extrapolation. For many chemicals, this is appropriate as there is either (1) evidence for low-dose linearity based on mechanistic data or epidemiological data or (2) no data suggesting a linear extrapolation is inappropriate. While this is a conservative and appropriate approach in these situations, it is not appropriate for situations where both mechanistic data and epidemiological evidence suggests less-than-linear extrapolations are warranted. Chloroform is a classic example where a threshold model for cancer was mechanistically supported; this model has since been incorporated into the U.S. EPA cancer potency for this chemical. A similar approach is now being implemented for dioxins. However, other chemicals with similar weight-of-evidence for non-linearity are so far being regulated using the linearized dose model for cancer was mechanistically supported; this model has since been incorporated into the U.S. EPA cancer potency for this chemical. A similar approach is now being implemented for dioxins. However, other chemicals with similar weight-of-evidence for non-linearity are so far being regulated using the linearized dose model.

1792 TOXICOLOGICAL PRINCIPLES FOR AN IMPROVED HAZARD NOTATION SYSTEM TO PROTECT WORKERS FROM DERMAL EXPOSURES.

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To alert workers and employers of potential health hazards arising from skin contact with chemicals the traditional practice has been to assign qualitative hazard designations called skin notations to indicate that a substance has the potential to be percutaneously absorbed, and thus affect the interpretation of inhalation-based occupational exposure limits. The National Institute for Occupational Safety and Health (NIOSH) has developed a new strategy for assigning skin notations capable of providing a warning beyond percutaneous absorption and to address the limitations associated with the historical approach used for assigning skin notations. The new strategy provides guidance for the systematic application of a weight-of-evidence approach. This includes critically evaluating available data (i.e., human, animal, in vivo, in vitro, and mathematical predictions) to assign multiple hazard-specific skin notations (SK) capable of clearly distinguishing between systemic effects, direct effects, and immune-mediated responses. This presentation will provide an overview of the new NIOSH strategy with emphasis on issues encountered during the evaluation of 140+ chemicals including: assigning a systemic effects notation where data or model predictions indicate absorption, but no or only limited dermal toxicity data are available; 2) differentiating among irritant severity levels when relying on qualitative studies that used different material dilutions and test systems; and 3) developing notations for sensitization when limited human studies and standard animal assays provide conflicting results. The lessons learned in evaluating such problematic data sets provide the basis for refining weight-of-evidence evaluation approaches for hazard notations.

1793 APPLYING THE MODERN PRINCIPLES OF RISK ASSESSMENT TO PROTECT WORKERS. UPDATE OF THE DERIVATION METHODS FOR IMMEDIATELY DANGEROUS TO LIFE AND HEALTH (IDLH) VALUES.

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The ability of airborne contaminants to quickly overwhelm victims has been well demonstrated within occupational settings resulting in acute and chronic irreversible health effects, and even death. Since the 1970s, the National Institute for Occupational Safety and Health (NIOSH) has been tasked with establishing acute exposure guidelines called Immediately Dangerous to Life and Health (IDLH) values to aid in protecting workers from such high risk environments. IDLH values are defined as: “atmospheric concentrations of toxic, corrosive, or asphyxiant substances that, via inhalation exposure, pose an immediate threat to life or would cause immediate or delayed irreversible adverse health effects or would interfere with an individual's ability to escape from a dangerous atmosphere in the event of a respirator failure.” NIOSH is in the process of revising the derivation process for IDLH values to ensure that they are sufficiently health protective. The objective of this presentation is to discuss the impact of a refined weight of evidence approach based on the modern principles of risk assessment for the derivation of new and revised IDLH values. The refined approach was applied to 20 case study chemicals; lessons learned from these case studies were used to hone the revised derivation method for IDLH values.

1794 ISSUES RELATED TO THE APPLICATION OF THE GHS STOT CRITERIA TO INHALED POORLY SOLUBLE PARTICULATES (PSP) OF LOW TOXICITY.

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The UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS) include hazard classifications addressing specific target organ toxicity (STOT) after acute or repeated exposure to chemical substances. Specifically, GHS provides the criteria as well as guideline values for the classification of particulates that are considered to produce specific target organ toxicity following repeated inhalatory exposure as Category 1 (produces significant toxicity in humans’) or 2 (‘harmful to human health’). To date, much of the data on the respiratory effects of inhaled particles comes from rat inhalation studies. The rat has, however, been shown to be more sensitive than humans or other rodent species to
exposure to particulates because of their tendency to impair lung clearance mechanisms leading to an overloading of the rat lungs at high particulate concentrations. This investigation examines and discusses on the basis of three case studies, whether the STOT-RE criteria and guideline values were applied to 3 different particulate materials considered as poorly soluble particles considered of low toxicity (PSP) still fit the original purpose of the classification. Subchronic exposure to PSP at concentrations below the GHS STOT-RE guideline values can lead to overload-dependent inflammatory responses in the rat that has only little relevance to humans. The lack of consideration of the rat’s unique sensitivity to PSP exposure has the potential to lead to STOT-RE category 1 or 2 classification for virtually any particulate material and will not distinguish between chemical-specific and particle-induced toxicity. Worker exposure to non-specific dusts is generally managed through occupational exposure limits. An inappropriate classification for STOT-RE for PSP would undermine the objective of the classification scheme.

The Globally Harmonized System of Classification and Labelling (GHS) and the European Union’s REACH Regulation (Registration, Evaluation, and Authorization of Chemicals) require a higher resolution of hazard classification than current U.S. regulations. They also require a concise but complete and comprehensible description of various health effects, symptoms, and chemical interactions, as well as the data used to identify these effects; this information is to be provided in Section 11 (Toxicological Information) of the Safety Data Sheet (SDS). The requirements include providing positive and negative human or animal data on the mixture or its hazardous ingredients, as well as indicating when no data are available. Information on dose, routes of exposure, symptoms, and chemical interactions is also required in Section 11. The dose-response information can inform the risk assessments required by REACH. These requirements can be challenging for a large, diverse, decentralized, multinational company that conducts business in more than 30 languages. In addition, these requirements must be met within the confines of existing data management systems. In order to meet these regulatory requirements, 3M is using standardized tools and processes to author and internally peer review health hazard profiles (HHPs) for the chemicals that it uses. The HHP collects information that can be used for the high-resolution hazard classification required by GHS and REACH and for other health hazard and risk assessments. Changes to our data management systems are being made to allow the extraction from the HHP of fielded (and therefore more easily translatable) toxicology data on a mixture and its components and its printing in Section 11 of the SDS. In order to meet the requirements of GHS and REACH, toxicologists are involved in regulatory interpretation, hazard assessment and classification, risk assessment, and systems development.

MEETING GHS AND REACH SAFETY DATA SHEET TOXICOLOGY REQUIREMENTS.


The Global Product Strategy (GPS) was launched by the International Council of Chemical Associations (ICCA) at the first International Conference on Chemical Management in 2006. GPS intends to improve the product stewardship components of the industry’s Responsible Care program. These two complementary initiatives are dedicated to the responsible handling of chemicals throughout the world and contribute to achieving the vision of United Nations Environment Program’s Strategic Approach to International Chemicals Management. Through implementing GPS and complying with regulatory requirements, companies belonging to ICCA will work to improve risk characterization and management procedures. It is envisaged that chemicals management is best accomplished through a combination of regulatory initiatives and meaningful voluntary industry programs. Under the GPS, by 2020, ICCA aims to 1) establish a base set of hazard and exposure assessment data to support the requirements for a base set element should follow a tiered approach where the need for additional testing is based on the hazard potential of the substance and the potential for human exposure. The presentation will expand on the proposed framework for the gathering and sharing of safety information to implement the GPS.
1799 GUIDANCE ON THE APPLICATION OF GHS (GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING) CRITERIA TO PETROLEUM SUBSTANCES.

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Classification and labelling of petroleum substances is not straightforward due to their complex and variable composition. Consistent application of GHS criteria requires an understanding of the influence of refining processes on the composition of various process streams as well as an understanding of the physico-chemical similarities of petroleum streams. IPIECA aimed to develop guidance which would complement the GHS and facilitate consistent classification of petroleum substances, resulting in appropriate hazard communication internationally. The guidance developed by IPIECA clarifies that while petroleum streams contain multiple hydrocarbon constituents, they are in fact unique substances with specific CAS numbers and descriptions, and are consistent with GHS and national regulatory definitions of substances, not mixtures. Petroleum substances are produced to meet physico-chemical specifications related to their intended use. Petroleum substances derived from similar starting materials, having similar physico-chemical properties and generally similar chemical composition, exhibit broadly similar hazard properties. These similar substances can be grouped for classification purposes. This 'category' classification approach facilitates full use of available data and minimises the need for animal testing. Where test data are available for a petroleum substance, the classification of the substance should be based on these data. In the absence of test data on the petroleum substance itself, read-across from a similar petroleum substance should be applied. In the absence of data on the substance or read-across data of similar substances, hazardous constituents should be considered in classification decisions per GHS guidance. It is concluded that the guidance provides a science-based, transparent approach to the classification of petroleum substances.

1800 EVALUATING HUMAN DATA FOR REACH: PROPOSED RELIABILITY SCORING SYSTEM FOR NICKEL COMPOUNDS.

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As part of the registration process under the Registration, Evaluation & Authorization and Restriction of Chemical (REACH) substances initiative, (eco)toxicity information must be compiled, scored for reliability, and synthesized for use in technical dossiers. One component of the process is submission of a Technical Dossier (as an output from an IUCLID database file) containing data for exposure related observations in humans, including epidemiological, sensitization, and direct measurements of exposure. Traditional Klimisch scoring for reliability and relevance are not sufficient for these types of studies. Thus a systematic approach based on key study design, analysis, and reporting criteria were developed for nickel compounds using a scoring system for lead risk assessment as a foundation. These criteria were tailored to address issues associated with evaluation of nickel and process streams as well as an understanding of the physico-chemical properties relevant to classification. As this approach has been used for nickel compounds, it has been applied to other compounds including chromium, cobalt, and copper.

1802 IDENTIFICATION OF FACTORS CONTRIBUTING TO THE DETECTION OF TEST ARTICLE IN CONTROL BIOANALYTICAL SAMPLES.

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The detection of measurable amounts of test article in bioanalytical samples from animals given control material presents a significant problem for both the contract research organization performing the study and the sponsor submitting the results to the appropriate regulatory agency. In 2005, the EMEA produced a guidance document, Guideline on the Evaluation of Control Samples in Nonclinical Safety Studies: Checking for Contamination with the Test Substance, which addressed these concerns and provided the following recommendations. 1) Assay control samples from all pivotal studies and all studies of administration. 2) Investigate and, if possible, identify the source contamination. 3) Properly document and assess the impact to the study. Importantly, the EMEA guidance document notes that relevant levels of test material in control samples have ultimately resulted in the invalidation for some studies, namely carcinogenicity and reproductive studies.

In this work, over 70 studies spanning ~4 years and containing ~7100 bioanalytical samples were evaluated for potential factors contributing to the contamination of control samples. Factors considered were dose route, species, batch, concentration, cell type, dose volume, matrix, and sample collection. The relationship of each parameter to the number of control samples and the number of control samples with contamination was assessed. Results indicate that while no single factor is solely responsibly for control sample contamination, species and type of molecule have considerable influence on the total number of control samples contaminated. Additional results such as bioanalytical method and dose route support the data surrounding molecule size.

1803 CONSIDERATIONS ON ADDRESSING THE SAFETY OF NON-ABSORBABLE POLYMER EXCIPIENTS.

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Many types of high molecular weight polymers are used as excipients in oral drug products, examples of which include poloxamers, polyethylene glycols, and poly(meth)acrylates. These polymers are essentially non-absorbable. However, as with any other oral excipient, many factors must be taken into consideration when assessing the potential risks associated with their use including exposure level and duration, local effects in the gastrointestinal system, and the potential for absorption and systemic effects. Established methods to derive safe oral exposure levels for humans typically involve applying safety factors (for species differences, study duration, etc.) to NOAELs from animal toxicology studies. However, application of this
methodology to derive safe exposure levels for non-absorbable polymers may not be practical due to relatively high human exposure, local site of contact effects, or lack of toxicity data. Some general guidance on adapting standard risk assessment methods to non-absorbable polymers is available, but there is no standard methodology. We suggest that due to their size and associated low potential for absorption, in general, the use of these methods to derive safe oral exposure limits for high molecular weight polymer excipients may be overly conservative. An alternative approach is proposed to assess the likelihood that an excipient of this type will exert unanticipated effects following oral administration. The focus is to determine safe levels of use in humans of these non-absorbable polymers that should not be associated with findings such as GI effects, physiological sequelae subsequent to phagocytosis, potential immune related effects, inflammation, or chronic irritation with potential tumorigenic responses. The use of this approach is demonstrated with representative excipients.

**1804 PREDICTIVE PERFORMANCE OF SOME STRUCTURE ACTIVITY TOOLS FOR IDENTIFICATION OF DERMAL SENSITIZERS IN PHARMACEUTICAL OPERATIONS.**

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The local lymph node assay (LLNA) is the preferred method in the EU, US, and other jurisdictions for identifying dermal sensitising potential of chemicals. As a prelude or adjunct to the LLNA the use of structure activity analysis (SAR) is recognized as making an important contribution, both to identify potentially hazardous materials prior to consideration of animal tests and in reducing the numbers of animals used in those tests. This study was conducted to evaluate the performance of two knowledge-based systems, DEREK (Lhasa Ltd, UK) and OECD QSAR Toolbox in the prediction of skin sensitisation, by comparing the output of each model to GSK LLNA data for 249 chemicals representative of pharmaceutical substances and routes of manufacture. Generation of structural alerts for dermal sensitization in DEREK compared with LLNA results showed overall concordance of 63%. For predictions of protein reactivity from OECD Toolbox compared to LLNA results agreement was 53%. Compared to LLNA results, both computer programs gave numerous false positive predictions, however negative predictivity was very good at 77% and 70% (DEREK and OECD Toolbox, respectively) suggesting future development may offer potential for exempting some chemicals from testing. Further assessment identified a series of commonly occurring alerts in the two systems and their respective performance was investigated. The better performing alerts tended to be certain alkylating agents such as haloalkanes (concordance 58%), or nucleophilic substitution of aromatics (concordance 67%). Other common alerts, e.g., aldehyde precursor (concordance 27%), or nucleophilic addition to ketones (concordance 14%) performed relatively poorly. Additional assessment might include modifying predictions with other considerations, e.g., weight of evidence approach using physico-chemical properties and other measures of biological reactivity such as results from the Ames test as a means to improve performance of SAR tools.

**1805 REDUCTION IN ANIMAL NUMBERS BY 17% IN NON-RODENT REPEAT DOSE TOXICOLOGY STUDIES WITHOUT COMPROMISING SCIENTIFIC QUALITY.**


In order to assess reversibility of treatment related changes in repeat-dose toxicology studies, it is necessary to include groups of animals retained for a specified period un-dosed at the completion of the dosing period. Within AstraZeneca this assessment is incorporated into the pivotal toxicology studies, typically one-month in duration, which support the first clinical trial in humans. Until now the one-month study design in dogs has included: Main study (n=24): 3M + 3F per group (control, low, mid, high) treated for one month + Recovery groups (n=12) 3M + 3F per group (control and high) treated for one month and left untreated for a further month. We have challenged the need for the recovery control group in our design. Control animals are fundamental for toxicological studies for data types that can only be collected once (e.g. pathology) by providing information on background changes as well as comparison for all treated groups. For data types collected repeatedly in the same individuals (e.g. ECG and Clinical pathology), each animal can act as its own control. Since the purpose of the recovery groups is to assess recovery from treatment related effects in the high dose main study animals, we believe that the only relevant comparison for this purpose is to compare high dose main study animals with high dose recovery animals. For this comparison no control recovery group is needed! The new study design is only possible, because of a relatively short recovery period, the use of mature animals (>10 months) and therefore a minimal risk of major age related shifts in background pathology. Lastly the pathology group uses a rotating current control from recent studies in order to aid in the identification of low incidence findings and non treatment related environmental factors. The decision to remove the recovery control group from one month toxicology studies has reduced the number of dogs by 6 to 30 per study without compromising scientific quality.

**1806 IMPACT OF REDUCING THE SAMPLE SIZE ON THE PERFORMANCE OF THE LLNA.**

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Allergic contact dermatitis (ACD) is an adverse health effect that results in lost workdays and can significantly diminish quality of life. To minimize the occurrence of ACD, regulatory authorities require testing to identify substances that may cause skin sensitization. The local lymph node assay (LLNA) is an alternative test method that virtually eliminates pain and distress associated with testing of substances for skin sensitization potential. OECD Test Guidance 428, which describes the LLNA, includes a requirement of at least four mice per group if the lymph nodes from all mice in the treatment group are pooled, and a minimum of five mice per group if the lymph nodes from each mouse are processed separately. To determine if data collected from four individual animals would suffice, NICEATM used data from LLNA tests (275 tests) to empirically determine the impact on the LLNA outcome of reducing the number of mice in each group from five to four. The average likelihood of agreement [both stimulation index (SI) < 3 or both SI ≥ 3] between LLNA outcomes with either four or five mice per group was 97.5% for the 275 treated groups. When comparing results on a test-by-test basis, there was complete agreement between outcomes with four or five mice per group for 90% (75/83) of the tests. For the remaining eight tests, there were some differences in classification between five and four mice samples, with the overall agreement averaging 83%. Much of the disagreement was due to the closeness of the SI to three, not to the reduction in sample size. The practical impact of reducing the sample size from five to four mice per group on the interpretation of experimental results appears to be minimal and, therefore, using four rather than five mice per group would not impact the overall performance of the LLNA for identifying potential skin sensitizers. NICEATM and ICCVAM have recently submitted a proposal to OECD to recommend that TG 429 be updated to include a requirement of a minimum of four animals per group. ILS staff supported by NIHES contract N01-ES-35504.

**1807 USING THE MURINE LOCAL LYMPH NODE ASSAY TO CATEGORIZE STRONG SKIN SENSITIZERS.**

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According to the U.S. Bureau of Labor Statistics, allergic contact dermatitis (ACD) is one of the most common types of occupational disease. Because the prognosis of ACD is poor, prevention is imperative. Criteria have recently been adopted to distinguish strong sensitizers from other sensitizers based on human, guinea pig, and LLNA data. Substances with positive responses in the human maximization test (HMT) or human repeat insult patch test (HRPT) at induction thresholds ≤500 µg/cm2 are classified as strong sensitizers. Similarly, LLNA EC3 values ≤2% are proposed to categorize substances as strong sensitizers and LLNA EC3 values >2% to categorize substances as "other sensitizers". In order to evaluate the accuracy of the LLNA for identifying strong sensitizers as defined by human data, NICEATM and ICCVAM used a database of 112 substances with both LLNA and human data to calculate human potency classification categories (strong vs. other than strong) predicted by various EC3 values. Classifications based on EC3 values were compared to those defined by several different threshold values derived from HMT and HRPT studies. Based on the available database, 64% of strong human sensitizers were correctly predicted using LLNA EC3 ≤2%, while the remaining 36% of strong sensitizers were underclassified as "other sensitizers". The current database indicates that over 1/3 of strong sensitizers would be underclassified as weaker skin sensitizers if the LLNA is used to determine potency categories. The LLNA should not be considered as a stand-alone test to predict skin sensitization potency. The LLNA EC3+ 2% can be used to categorize a substance as a strong sensitizer.
However, substances with EC3 values greater than 2% are not necessarily moderate or weaker sensitizers. The LLNA should be used in an integrated decision strategy (e.g., with QSARs, peptide reactivity, human evidence, and historical data from other animal studies) to discriminate between strong and other skin sensitizers. ILS staff supported by NIEHS contract N01-ES-35504.

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1808 IMPLEMENTATION OF THE NICEATM-ICCVAM FIVE-YEAR PLAN: ADVANCING THE DEVELOPMENT, VALIDATION, ACCEPTANCE, AND APPROPRIATE USE OF ALTERNATIVE TEST METHODS.

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Safety testing of chemicals, consumer products, and other substances is necessary to prevent injury and disease by identifying potential health hazards and ensuring proper hazard classification and labeling. ICCVAM’s mission is to facilitate the development, validation, and regulatory acceptance of alternative safety test methods that protect human and animal health and the environment while reducing, refining, and replacing animal use. NICEATM and ICCVAM developed a Five-Year Plan in conjunction with its 15 member agencies that builds on the ICCVAM mission to achieve progress and to inform the public of their strategy. An overall goal of this plan is for ICCVAM to assume a greater leadership role in promoting research, development, translation, validation, and regulatory acceptance of alternative test methods. A working document has now been developed to describe how the strategies outlined in the Five-Year Plan are being implemented. Implementation activities address four key challenges: 1) identifying test method priorities and conducting and facilitating activities in these areas; 2) identifying and promoting new science and technology; 3) fostering regulatory acceptance and use of alternative test methods; and 4) developing partnerships. This plan is predicated on a proactive role for NICEATM and ICCVAM to identify and develop collaborations with experienced scientists that can bring state-of-the-art science to the forefront. This will require working closely with a broad range of stakeholders because ICCVAM, as an interagency committee, does not have resources to conduct research, development, and validation studies. Therefore, successful implementation will depend on these interactions both within and outside of ICCVAM agencies. ILS staff supported by NIEHS contract N01-ES-35504.

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1809 IS THE RECENT JECAF ADI FOR PHYTOSTEROLS, PHYTOSTANOLS, AND THEIR ESTERS UNNECESSARILY RESTRICTIVE?


Phytosterols, phytostanols, and their esters have recently been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECAF), who established a group acceptable daily intake (ADI) of 0 to 40 mg/kg body weight/day (approximately 2.4 g/day) based on an overall No-Observed-Adverse-Effect-Level (NOAEL) of 4,200 mg/kg body weight/day from several short-term toxicity studies. Significant increases in cardiomyopathy and decreases in body weight gains reported in rodents receiving a considerably higher dose of phytosterol esters (9,000 mg/kg body weight/day) were considered by JECAF to be adverse effects, and therefore reclassified as a Lowest-Observable-Effect-Level (LOAEL). However, a thorough review of the study design and adverse effects reported by the authors revealed several limitations that suggest the findings of this study are not relevant to humans consuming phytosterol enriched foods. A conclusion that the findings of this study are not relevant to dietary phytosterol consumption is important as we have noted that the phytosterol intakes reported in the study were incorrectly translated by JECA during their evaluation, an error that has significant implication to JECAF’s ADI derivation. Our findings are significant for two reasons. First, contrary to the prevailing view that maximal reduction in serum cholesterol is obtained from dietary intakes of 2 to 5 g/person/day, new clinical evidence indicates that the consumption of up to 9 g phytosterols reduces low density lipoprotein-cholesterol levels beyond those reductions achievable with doses that are in line with the JECAF ADI. Secondly, the current ADI makes it virtually impossible to fortify foods in a manner that provides efficacious quantities to median users without exceeding the ADI in frequent consumers of these products. We have therefore concluded that the current body of scientific evidence pertaining to the safety of phytosterol/sterol enrichment of food products does not provide a basis for setting an upper limit to total daily intake of phytosterols.

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1810 RETROSPECTIVE REVIEW OF CARDIOVASCULAR FINDINGS FROM REPEAT-DOSE TOXICITY STUDIES IN BEAGLE DOGS.

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Electrocardiogram (ECG) measurements are included in the design of repeat-dose toxicity studies in non-rodents and are obtained at baseline and at selected time points during the dosing and recovery phases. These measurements are usually timed to examine potential effects when peak test article plasma concentration occurs (Tmax). The integration of cardiovascular (CV) endpoints in repeat dose toxicology studies requires extensive technical resources and interpretation of results can be confounded by the high dosages utilized in these studies. For regulatory filings, CV safety pharmacology is routinely assessed in stand alone studies in accordance with ICH S7A guidance. In addition to an assessment of electrocardiographic function, these studies also provide assessments of blood pressures. Moreover, CV safety pharmacology studies are conducted in telemetered animals, therefore assessing of a lowing continuous analysis while avoiding issues with other confounding factors due to animal handling. Regulatory guidelines, including the ICH M3 (R2) (2009), OECD 409 (1998), and OECD 452 (1981) do not specify the need to perform ECG evaluations in repeat-dose toxicity studies; however, the CPMP guidance on repeat dose toxicity studies does call for them. The aim of this study is to review ECG results from repeat-dose toxicity studies in Beagle dogs (1- to 9-months in duration) conducted by Abbott from 2002-2009 and compare them with single-dose CV safety pharmacology studies in dogs during the same interval. A review of studies supporting 17 compounds has been completed to date and no repeat-dose toxicity studies with physiologically relevant cardiovascular findings have been identified. Based on the results of these and further comparisons, the intent is to propose, a reduction or elimination of CV assessments in repeat-dose toxicity studies and to generate a dialogue with the other industry toxicologists, as well as regulators, who are responsible for generating and/or assessing the data from these studies.

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1810A ESTABLISHMENT OF THE INTERNATIONAL COOPERATION ON ALTERNATIVE TEST METHODS (ICATM) AND ITS ROLE IN THE VALIDATION AND REGULATORY ACCEPTANCE OF GLOBALLY HARMONIZED SAFETY ASSESSMENT METHODS.

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Several countries have established national validation organizations to promote the validation, evaluation, and regulatory acceptance of alternative safety testing methods that may reduce, refine, and replace the use of animals while maintaining adequate protection of human and animal health and the environment. On April 27, 2009, Canada, the European Union, Japan, and the U.S. signed a Memorandum of Cooperation (MOG) on International Cooperation on Alternative Test Methods (ICATM) to promote enhanced cooperation, collaboration, and communication among their respective validation organizations. The initial participating validation organizations are the Environmental Health Science and Research Bureau within Health Canada, ECVAM, JaCVAM, and ICCVAM-NICEATM. The organizations developed an initial framework to promote harmonization of scientific recommendations on alternative toxicity testing methods in response to a charge from the International Cooperation on Cosmetics Regulation. The ICATM MOG implements this framework and lays out processes for cooperation across three critical areas: design and validation studies, independent scientific peer review, and development of harmonized test method recommendations for regulatory consideration. This agreement is providing greater efficiency and effectiveness for the participating validation organizations by avoiding duplication of effort and leveraging of resources for recently initiated international validation studies. The integrated cooperation and harmonization is expected to support more rapid international adoption of scientifically valid test methods by organizations such as the OECD that will protect people, animals, and the environment while reducing, refining, and replacing animal use where scientifically feasible.

Several different metal compounds have been identified as carcinogens. Recent studies have shown that metalloproteins (MMPs) were secreted enzymes selectively degrading the extracellular matrix and had been implicated in tumor cell invasion. Therefore, we have carried out a study on the effects of a series of metals (Cd²⁺, Cr⁶⁺, Cu²⁺, Fe³⁺, Ni²⁺ and Zn²⁺) on secretion of MMP from mouse embryonic fibroblast cells, immortalized by SV40 large T-antigen. In zymographic analysis, high level of 68kDa latent enzyme of MMP2 was secreted in the conditioned medium of the cells. The cells were exposed to the metals for 24 hours and conditioned medium was collected for zymographic analysis. Secretion of MMP2 from the cells was found to be strongly inhibited by several metal ions. Cd²⁺ was the most effective followed by Zn²⁺ and Cr⁶⁺. Treatment of 0.1 μM Cd²⁺ significantly inhibited the secretion of MMP2 by up to 60% and 30%, respectively. However, Cd²⁺ did not affect MMP2 gene expression. These data indicated that Cd²⁺ inhibited secretion of MMP2 from the cells without decrease of MMP2 synthesis. Invasiveness of the cell to reconstituted basement membrane Matrigel was significantly inhibited by 0.5μM Cd²⁺ for 24 hours. These results indicate that Cd²⁺ inhibits cell invasion by decreasing secretion of MMP2 from cells. Unexpectedly, Cd²⁺ appeared to be an effective anti-metastasis in this system. (Supported by a Grant-in-aid for General Research from the Ministry of Education, Sciences, Sports and Culture of Japan.)

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Metallothioneins (MTs) are low molecular weight, cysteine-rich metal binding proteins that play central roles in metal homeostasis. Metal-inducible MT transcription is regulated by the interaction between the cis regulatory metal responsive element (MRE) and the MRE-binding transcription factor MTF-1 (metal-regulatory transcription factor-1). The mechanism by which metal activates MTF-1/MRE-mediated transcription is not yet fully resolved. We propose a model in which the regulation of MT transcription is controlled by signal transduction cascades that activate MTF-1 phosphorylation, which include PKC and CK2. We investigated the role of potential PKC and CK2 phosphorylation sites using site-directed mutagenesis of MTF-1 and lentiviral transduction of dko7 cells (MTF-1 null murine embryonic fibroblasts). We identified two PKC consensus sites, T224 and S641, where phosphorylation of MTF-1 was detected after exposure to cadmium or zinc. Consistent with this finding, whole cell extracts prepared from cells expressing wild-type MTF-1 after exposure to cadmium or zinc yielded levels of MT-I and MT-II metal induction similar to cells transduced with lentiviral MTF-1. Deletion of the C-terminal cysteine cluster or mutation of the cysteine residues in the C-terminal domain of MTF-1 abolished or markedly reduced the MT transcription activity compared to wild-type MTF-1.

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Metallothionein family is involved in the maintenance of vectorial active ion transport in cultures of human proximal tubule (HPT) cells. We have demonstrated that MT-3 is involved in regulation of cell differentiation by controlling the epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET). Recent in vitro and in vivo studies have reported that MT-3 is interacting with other proteins and these interactions are thought to be important in regulation of some of its functions. The goal of our study is to identify the binding partners of MT-3, which may allow us to understand the mechanism through which MT-3 is regulating the vectorial active transport and cell differentiation in HPT cells. We performed MT-3 pull-down experiments followed by SDS-PAGE and mass spectrometry analysis in an immortalized human proximal tubule cell line, HK-2 cell extract and renal cortical tissue extract. We have identified β-actin, tropomyosin, gelsolin and myosin (non-muscle) as the binding partners of MT-3 and rather than interacting with MT-3, these proteins are found in the complexes that contain the above mentioned proteins. These studies demonstrate that MT-3 is interacting with the proteins that are involved in cytoskeleton reorganization of the cell and thereby regulating the vectorial active transport and cell differentiation.

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Metallothioneins (MTs) belong to a superfAMILY of intracellular proteins that sequester environmentally toxic metals and are thought to regulate their intracellular concentration. MT expression is transcriptionally regulated by the metal-regulatory transcription factor 1 (MTF1). MTF1 acts by binding to short DNA sequences in the enhancer/promoter regions of target genes including MT-I and MT-II. To examine the role of phosphorylation in MTF1 transcriptional activation, PKC consensus sites were identified within MT-I and each site was systematically mutated. Lentivirus were developed for each mutant, transduced into MTF1 knockout mouse embryonic fibroblasts (dko7) cells and stable cell lines expressing each construct were isolated. We exposed these cells to cadmium or zinc, and measured changes in expression of two marker genes: MT-I and MT-II, by qPCR and Western Blot analysis. Deletion of the C-terminal cysteine cluster or mutation of the cysteine residues abolished or markedly reduced the MT transcription activation activity of MTF1 and the ability of MTF1 to restore MT-I induction in MTF-1 KO cells. The findings demonstrate a critical role of the C-terminal cysteine cluster of MTF1 in arsenic sensing and gene transcription via arsenic-cysteine thiol interaction.

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CD-INDUCED EGFR TRANSACTION LEADS TO STAT3 ACTIVATION BY A MECHANISM INVOLVING PKC, SRC, AND NADPH OXIDASE.


Humans are susceptible to Cd ion toxicity. Following pulmonary or gastrointestinal absorption, Cd is taken up by the liver where it forms complexes with small peptides, including glutathione or the high affinity metal binding protein metallothionein (MT). Cd induces NADPH oxidase activity, which produces reactive oxygen species (ROS). Increased phosphorylation of signal transducer and activator of transcription 3 (STAT3) has been associated with Src and the epidermal growth factor receptor (EGFR) resulting in ERK activation. Our objective was to evaluate the participation of EGFR/Src and PKC/NADPH oxidase in STAT3 activation in mouse hepatocytes treated with 5 μM CdCl2. Hepatocytes were treated with Cd for different periods and STAT3, and ERK activation and MT content were determined by Western blot. A pretreatment with EGFR inhibitor AG1478, Src inhibitor SU6656, PKC inhibitor Chelerythrine or NADPH oxidase inhibitor AEBSF were assessed in STAT3 activation. NADPH oxidase and PKC were inhibited and ROS generation was determined with DCFH-DA. Cd increased STAT3 and ERK1/2 activation. STAT3 activation decreased in presence of EGFR, Src, PKC, and NADPH oxidase inhibitors, PKC and NADPH oxidase contribute in Cd-induced ROS generation. MT increased as a result of Cd treatment in a time-dependent manner. Our results suggest that Cd activates STAT3 via EGFR/Src and PKC/NADPH oxidase pathway producing ROS that increase ERK1/2 phosphorylation in a PKC-dependent manner that probably confer protection against Cd injury in hepatocytes. SEP-CONACYT, CB-2006-1-6081 y CONACYT: 20473 y 229382/212789.

LOW-LEVEL CADMIUM EXPOSURES DO NOT ALTER INTRACELLULAR CALCIUM LEVELS.

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Cadmium constitutes a threat to human health. However, the molecular mechanisms underlying cadmium-regulated transcription remain unclear. It has been proposed that modulation of intracellular calcium levels (Ca2+i) following cadmium exposure may be one mechanism by which cadmium can affect transcription. To test this, we assessed the effects of cadmium exposure on calcium homeostasis and calcium signaling in HEK293 cells expressing a protein-based calcium ion sensor, cameleon YC 3.60. We found that exposing HEK293 cells to 1 μM cadmium for 4 h, a non-toxic concentration sufficient to induce transcription of cadmium-responsive genes, had no effect on [Ca2+]i mobilization. We also assessed the effects of cadmium on calcium-responsive genes and signaling, and found that only toxic concentrations of cadmium (>LD50) were capable of altering transcriptional activity. To gain further insights into the mechanistic relationship between cadmium and calcium, we investigated the effects of cadmium on IP3–dependent Ca2+ signaling in the nematode Caenorhabditis elegans. IP3–dependent Ca2+ signaling regulates the defecation cycle in C. elegans. Low-level cadmium exposures were found to shorten the defecation cycle length but did not alter the rhythm or the magnitude of the calcium waves. In contrast, C. elegans exposed to the SERCA pump inhibitor, thapsigargin, exhibited defecation cycles that were significantly lengthened and highly arrhythmic. The effect of cadmium exposure on the rhythmic defecation cycle resembles a phenotype observed in C. elegans mutants that are not capable of proper lipid synthesis. This suggests that cadmium’s effect on defecation may not be due to alterations in IP3–dependent Ca2+ signaling but rather by effects on lipid synthesis. In summary, our data indicate that the effect of cadmium exposure on gene transcription in HEK293 cells and the defecation process in C. elegans are independent of [Ca2+]i mobilization.

ROLE OF THE CYTOSKELETON IN CADMIUM-INDUCED DEATH OF MOUSE MESANGIAL CELLS.

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BACKGROUND: Cadmium induces cell death in rodent mesangial cells (MC) that is part dependent on reactive oxygen species (ROS) and activation of multiple kinases, including the Ca2+-calmodulin-dependent kinase II (CaM-KII) and p38 kinase. It also leads to disruption of the actin cytoskeleton. Because the cytoskeleton plays an important role in several cellular functions, including both apoptosis and cell survival, we investigated the role of the cytoskeleton in Cd2+-induced cell death. METHODS: Cultured MC were prepared from out-growth of glomeruli isolated from kidneys of adult mice. MC were treated in serum-free conditions with 10 μM CdCl2, in the presence or absence of inhibitors, with or without jasplakinolide (a cytoskeletal stabilizer) or cytochalasin D (a cytoskeletal disruptor). Cell populations were examined by flow cytometry following staining with FITC–Annexin V and propidium iodide (PI), distinguishing Annexin V +ve populations of apoptotic (PI–ve) and apoptotic-like (PI+ve) cells, as described (J. Cell. Physiol. 217: 307-318 (2008)). RESULTS: Cadmium decreased cell viability and increased both apoptotic and apoptotic-like death. Disruption of actin filaments with cytochalasin D was partially reversed, whereas stabilization with jasplakinolide was without effect, indicating that cytoskeletal disruption contributes, but is not necessary for, induction of apoptosis. Activation of CaM-KII and p38 kinase, and treatment with the antioxidant N-acetyl cysteine, all improved cell viability. Whereas the effect of kinase inhibition was on apoptosis, the effect of antioxidants was primarily on apoptotic-like death. However, all protected against disruption of the cytoskeleton. Cytochalasin D decreased Cd2+-dependent ROS production and decreased phosphorylation of p38 kinase. CONCLUSIONS: Cd2+-dependent actin disruption is a downstream event that facilitates apoptotic death. This cell death involves actin-dependent mitochondrial changes, ROS production, and p38 activation.

CYTOTOXIC AND GENOTOXIC EFFECTS OF COEXPOSURE TO NICKEL AND CADMIUM.

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Nickle and cadmium are well known human carcinogens which can trigger carcinogenicity in cells through a variety of mechanisms including: increased DNA damage, inhibition of DNA repair mechanisms and enhanced genotoxicity. Single exposure to NiCl2 or CdCl2 exhibits a dose-dependent increase in cell apoptosis which is only weakly mutagenic. It is not until these metals reach relatively high doses that they cause DNA damage in cells. Most studies have investigated the effects of single metal exposure while this study examines the combined cytotoxic and genotoxic effects of coexposure using a human cell culture model. We exposed human lung epithelial cells to NiCl2 and CdCl2 for 24 hr and monitored cell viability, apoptosis, and micronuclear formation. We found increased clonogenic lethal- and apoptosis as seen by enhanced activity of caspase 3 and 7 and cleavage of PARP. In single and dual exposures no change in p53 or XPA expression was observed suggesting NiCl2 and CdCl2 induced toxicity occurs in a p53- and XPA-independent manner. Micronuclear assays found that single NiCl2 was not genotoxic while in combination with CdCl2 it increased mutation frequency. Collectively, this data demonstrates that exposure to multiple metals simultaneously results in enhanced cytotoxicity and genotoxicity.

CADMIUM EXPOSURE LEADS TO ERK ACTIVATION IN THE HUMAN OSTEOSTAT-LIKE CELL LINE, SAOS-2.

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Cadmium is a heavy metal associated with the development of several bone diseases including osteoporosis. Apoptosis and oxidative stress are mechanisms involved in the pathogenesis of osteoporosis. We previously reported that cadmium induces apoptosis and oxidative stress in human osteoblast-like cell line, Saos-2. The aim of our study is to elucidate the cell signaling pathways involved in cadmium’s induction of apoptosis in Saos-2 cells by examining PKC (Protein Kinase C) and ERK (Extracellular Signal-Regulated Kinase). We hypothesize that the PKC and ERK pathways are up-regulated in the presence of CdCl2, and predict that treatment with a PKC or ERK inhibitor will prevent cadmium-induced apoptosis. Activation of PKCα and ERK protein was assessed by western blot in cells treated with 10 μM CdCl2 or control cultures for 1-4 hours. Apoptosis was determined using ApoPercentage dye that stains apoptotic cells. We found that pPKCα did not in- crease significantly in cells exposed to 10 μM CdCl2 compared to controls. When cells were pretreated with Calphostin C, a general PKC inhibitor, there was no sig- nificant change in the amount of apoptosis compared to CdCl2 alone. In contrast, pERK was up-regulated after 3 or 4 hour treatment with 10 μM CdCl2 compared to controls (3 Hour Control Relative Density pERK/ERK = 0.44 ± 0.11 and 3 hour treatment Relative Density pERK/ERK = 0.87 ± 0.01). These findings sug- gest that PKC is not involved in cadmium induced apoptosis. However, ERK acti- vation may be involved in cadmium’s toxicity. Ongoing studies aim to link ERK ac- tivation to cadmium-induced apoptosis. Elucidation of cellular pathways involved in cadmium-induced osteotoxicity will lend to the understanding of underlying mechanisms of human bone disease. Research funded by INBRE P20RR016454 and NIH R15ES015866 Grants.
1821 BLOOD AND URINARY CADMIUM CONCENTRATION OF RESIDENTS AROUND ABANDONED METAL MINES IN KOREA.

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The purpose of this study is to evaluate the blood and urinary cadmium concentration levels of the residents around the abandoned metal mines and the control group. Blood and urinary cadmium concentration levels were analyzed through investigations of the dietary habits and dietary water of subjects who live near abandoned metal mines (exposure group) and in the control group. These levels were different significantly between the exposure group and the control group. It was found that the findings of this study will in the future prevent further illness from this type of exposure.

1822 OVEREXPRESSION OF CDC34 OR UBC4, UBIQUITIN-CONJUGATING ENZYMES, CONFERS RESISTANCE TO CADMIUM THROUGH DIFFERENT MECHANISMS IN YEAST CELLS.

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Cadmium is an environmental pollutant of great concern because of its bioaccumulation and high toxicity that causes severe damage to kidneys. Although metallothionein is well known as a protein that is involved in bioprotection against cadmium toxicity in several organisms including humans and yeast cells, little is known about other mechanisms of bioprotection. We found that the overexpression of Cdc34 or Ubc4, ubiquitin-conjugating enzymes that are component of the ubiquitin-proteasome system, confer resistance to cadmium in yeast cells. The yeast cells overexpressing Cdc34 were resistant to cadmium even in the presence of the proteasome inhibitor MG132. However, the acquired resistance to cadmium by overexpression of Ubc4 was not observed in the presence of MG132. Moreover, overexpression of Cdc34 was shown to inactivate the transcriptional activity of Met4, which is specifically ubiquitinated by Cdc34, by accelerating its ubiquitination and to reduce expression of the MET25, a target gene of Met4. Unlike Cdc34, overexpression of Ubc4 did not affect the expression of the MET25. A MET25-deleted strain of yeast showed greater cadmium resistance than wild-type strains, but overexpression of Cdc34 in the MET25-deleted yeast did not affect cadmium sensitivity, suggesting that Met4 is essential for acquisition of cadmium resistance by overexpression of Cdc34. Met4p is an enhancer that catalyzes synthesis of homocysteine from S~12~ and O-acetylated homocysteine. Overexpression of Cdc34 decreased the expression of the MET25 gene and increased the cellular concentration of S~12~. These results suggested that overexpression of Cdc34 inactivates Met4p and reduces expression of the MET25 gene, leading increased production of low-toxic CdS. By contrast, the mechanism of acquired resistance to cadmium by overexpression of Ubc4 is different from that of Cdc34 and that Ubc4 confers resistance to cadmium by ubiquitination of proteins other than Met4 and accelerates the degradation of these proteins in the proteasome.

1823 LONG-TERM CADMIUM EXPOSURE RESULTS IN ENHANCED NITRIC OXIDE PRODUCTION FROM LPS-STIMULATED RAT SPLENOCYTES.

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It is uncertain if the presence of nitric oxide (NO) enhances or mitigates cadmium (Cd) toxicity. NO is essential for proper immune system function. However at high concentrations, NO can become a harmful oxidant. To examine how Cd may alter endogenous NO production, Sprague/Dawley rats were given subcutaneous injections of Cd in the form of CdCl2 at a dose of 0.6 mg/kg, 5 days per week for 12 weeks. Splenocytes isolated from 12 week Cd treated rats were found to produce significantly greater amounts of NO following exposure to 10, 100 or 1000 μg of lipopolysaccharide (LPS) in a dose dependent manner compared to control animals. Because NADPH is essential in NO production and the two enzymes, glucose 6 phosphate dehydrogenase (G6PDH) and 6 phosphogluconate dehydrogenase (6PGDH) are the main sources cellular of NADPH, further studies were conducted to examine the effects of Cd on the activity of G6PDH and 6PGDH. G6PDH activity was significantly higher in spleen tissue from 12 week Cd treated animals. Since the kidney is the main target of Cd toxicity, additional studies were performed to examine potential changes Cd may have on G6PDH and 6PGDH. Kidney tissue from Cd-treated animals had significantly greater activities of both G6PDH and 6PGDH and elevated expression of G6PDH. This study indicates that Cd may cause enzymatic changes resulting in enhanced LPS-stimulated NO production in the spleen and possibly other tissue. It is unclear what role the LPS-mediated increase in NO production may have in Cd toxicity. Support in part by Grant RO1ES006478 from the NIEHS.

1824 SUBCHRONIC CADMIUM EXPOSURE RESULTS IN DECREASED INSULIN SECRETION AND HYPERGLYCEMIA PRIOR TO ONSET OF RENAL DYSFUNCTION IN RATS.

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Several recent epidemiological studies have shown significant associations between exposure to environmental contaminant cadmium (Cd) and diabetes. These studies add compelling evidence that Cd exposure may be a contributing environmental factor for the development of diabetes. To further examine the diabetogenic effects of Cd, Sprague/Dawley rats were given subcutaneous injections of Cd in the form of CdCl2 at a dose of 0.6 mg/kg, 5 days per week for 12 weeks. Weekly urine samples and fasting blood glucose data were collected. Significant differences in fasting blood glucose levels from Cd treated control (vehicle sallined) vs Cd treated animals were detected several weeks prior to statistically significant changes of overt signs of renal dysfunction such as polyuria. Furthermore, at week 12 the mean fasting insulin value from Cd treated animals was significantly less (~50%) of that of control values. Examination of histological slides showed evidence that Cd caused retraction and separation of pancreatic β-cells. This could be especially significant because others have reported that a loss of cell-cell adhesion in pancreatic β-cells and the relocation of proteins associated with cell adhesion, specifically E-cadherin, results in decreased insulin release. Results from this study provide additional evidence that Cd exposure may be a contributing environmental factor for the development of diabetes. This study also suggests that disruption of cell-cell interaction in pancreatic β-cells may be an important mechanism of Cd-induced hyperglycemia and decreased insulin release. Supported in part by RO1ES006478 from the NIEHS.

1825 GENETIC EVIDENCE OF RESISTANCE TO CADMIUM-INDUCED TESTICULAR TOXICITY IN INBRED WISTAR-IMAMICHI RATS.


The toxic effect of cadmium (Cd) varies with species, strain and sex in experimental animals. We have previously demonstrated that Wistar-Imamichi (WI) rats are strongly resistant to Cd-induced lethality and hepatotoxicity compared to Fischer 344 (F344) rats. Since the testes are one of the most sensitive organs to acute Cd toxicity, we further examined strain-related difference in Cd-induced testicular toxicity between inbred WI and F344 rats. Cd at doses of 1.0 and 2.0 mg/kg, sc, induced severe testicular hemorrhage, as assessed by pathological changes and testicular hemoglobin level, in F344 rats, but did not in WI rats. When the animals were treated with Cd at a dose of 2.0 mg/kg, sc, the testicular content of Cd was significantly lower in WI rats than in F344 rats, indicating a toxiokinetic mechanism for the observed strain difference. So far, evidence has been provided that the resistance to Cd-induced testicular toxicity is genetically regulated in mice. However, no information on the genetic regulation of resistance to Cd-induced testicular toxicity is available in rats. Thus, we attempted a simple Mendelian genetic analysis of testicular hemoglobin levels, as an indicator of Cd (2.0 mg/kg, sc) induced testicular toxicity, by using the inbred WI and F344 rat strains. Male first filial (F1) generation progeny exhibited the testicular hemoglobin levels of intermediate type which approximate the mean of the two parental types, and male second filial (F2) generation progeny segregated into three types of low/intermediate/high in the frequency distribution of testicular hemoglobin levels. The ratio of low/intermediate/high was significantly different from 1:1. These results lead us to conclude that genetic background is the critical factor in determining the resistance to Cd-induced testicular toxicity in inbred WI rats.
KERATIN 7 EXPRESSION IN INDEPENDENT ISOLATES OF CADMIUM TRANSFORMED HUMAN UROTHELIAL CELLS (UROTSAs).

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This laboratory has shown that a human urothelial cell line (UROtsa) transformed by cadmium (Cd) produced subcutaneous tumor heterotransplants that resemble human transitional cell carcinoma (TCC). In the present study, additional Cd-transformed cell lines were isolated to determine if independent exposures of the cell line to Cd would result in malignantly transformed cell lines possessing similar phenotypic and genotypic properties. Seven independent isolates were isolated and assessed for their doubling times, morphology, ability to heterotransplant subcutaneously and in the peritoneal cavity of nude mice and for the expression of keratin 7. The 7 cell lines all displayed an epithelial morphology with no evidence of squamous differentiation. Doubling times were variable among the isolates, being significantly reduced or similar to the parental cells. All 7 isolates were able to form subcutaneous tumor heterotransplants with a TCC morphology and all heterotransplants displayed areas of squamous differentiation of the transitional cells. The degree of squamous differentiation varied among the isolates. In contrast to subcutaneous tumor formation, only 1 isolate of the Cd-transformed cells (UTCd#1) was able to effectively colonize multiple sites within the peritoneal cavity. An analysis of keratin 7 expression showed no correlation with squamous differentiation for the subcutaneous heterotransplants generated from the 7 cell lines. Keratin 7 was expressed in 6 of the 7 cell lines and their subcutaneous tumor heterotransplants. Keratin 7 was not expressed in the cell line that was able to form tumors within the peritoneal cavity. These results show that individual isolates of Cd-transformed cells have both similarities and differences in their phenotype and genotype.

CIGARETTE SMOKE CADMIUM INCREASES RELEASE OF CALCIUM 41 FROM BONE IN HUMANS.

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This project aims to contribute to the growing evidence that cadmium (Cd) is a significant and preventable cause of osteoporosis in humans. To date, studies of smoking effects on osteoporosis vary in their results. However, no osteoporosis study has stratified smokers according to their Cd exposure levels, and smokers vary as much as 10-fold in their blood Cd concentrations (CdB), depending on past smoking habits and tobacco leaf Cd concentrations. For this study, 20 postmenopausal women who smoke cigarettes and 10 non-smokers received a single intravenous administration of Ca-41 isotope. Starting 4 months later, 5 blood and 11 urine samples were collected over 7 weeks: 3 weeks when smokers smoked at their customary rate, the next 4 days when all smokers ceased smoking, the next 3 weeks when as many smokers as possible maintained a no-smoking status. Smoking status was documented with a daily diary and verified with a carbon monoxide breath test. Analyses included CdB values and serum levels of Ca-41, total Ca, and bone biomarkers. Bone mineral density (BMD) measurements were taken by heel ultrasound. Inhaled Cd was estimated using a smoking machine. Results demonstrated a 10-fold range of CdB values among smokers (0.3 to 3.0 ng Cd/ml), with the lowest value in range of those in non-smokers (0.2 to 0.6 ng Cd/ml). Starting at ~1.5 ng Cd/ml blood, Cd was associated with an increased release of Ca-41 from bone during the 4 days of smoking cessation. For those who resumed smoking after the 4-day period, Ca-41 release from bone was dependent on Cd status, with increased release in persons with higher CdB values. To illustrate, the person with the highest CdB value showed no decrease in Ca-41 release from bone over 7 weeks, while non-smokers and smokers with lower CdB values showed a 40% decrease. Finally, a negative correlation was observed among smokers between heel bone Z-score and CdB value (R2, 0.21). Identifying Cd as a preventable cause of osteoporosis is an important undertaking, analogous to identifying asbestos as a cause of lung cancer.

OVER-EXPRESSION OF HSULF-1 IN HUMAN LUNG EPITHELIAL CELLS ENHANCES THE TOXIC EFFECTS OF LEAD AND CADMIUM.


Alveolar epithelial cells, key components of the air exchange surface of the lung, must proliferate and differentiate quickly following injury to prevent irreversible fibrosis. Cadmium is known to damage alveolar epithelial cells and reduce proliferation and differentiation, resulting in fibrosis. Cell surface heparan sulfate proteoglycans (HSPGs) are believed to play important protective roles against injury. HSulf-1, an extracellular 6-O-endosulfatase that removes sulfate groups from HSPGs, could exacerbate cellular injury caused by exposure to metals either directly or by modifying signal transduction pathways. To examine this possibility, transformed human lung epithelial (H292) cells were transiently transfected with an adenovirally-delivered over-expression plasmid. After 48 hours cells were trypsinized, seeded, and treated in culture with cadmium chloride (10 - 70 μM) or lead nitrate (32 – 2,048 μM). At the end of 48 hours, over-expression of HSulf-1 specifically increased inhibition of cell proliferation and cell death when treated with cadmium or lead treatments compared to cadmium or lead alone. In addition, PCR array indicated that TGF/BMP signaling pathway activation by cadmium treatments at 15 μM was reduced by over-expression of HSulf-1. These findings suggest that HSulf-1 functions to modify injury induced by different exposure levels of cadmium and lead, and that sulfate groups on HSPGs play an important role in protection against environmental toxicants.

SEVERAL METAL COMPOUNDS AFFECT HUMAN PERIOD GENE EXPRESSION LEVELS.


Circadian rhythms in mammals are generated by two interconnected feedback loops that drive the rhythmic expression of a set of clock genes. These genes and gene products are related to various biological functions such as cell proliferation, apoptosis, hypoxia and hormonal excretion in addition to rhythmic formation. Over-exposure to mammalian cells with metals including Cd, a typical environmental and industrial pollutant, show inhibitory effect on the similar biological events that clock genes rela.te. Period (Per) genes are one of the master regulators of clock genes. To assess whether the Per genes are toxicological targets of metals, this study examined the effect of metal exposure on expression levels of Per genes. HeLa cells were exposed to CdSO4, ZnSO4 or CuSO4 for 6 hr followed by RNA isolation. Expression levels of Per1, Per2 and Per3 genes were analyzed by real-time PCR using Lightcycler 480 system. Cd markedly down-regulated Per1 and Per2 expression levels at 5 μM; this concentration slightly reduced the cell viability (about 90%). At higher doses, these gene's expression levels were somewhat rose. Zn and Cu showed no (or slightly) inhibitory effect on Per1/2 expressions, however, induced Per3 expression level adversely. These down-regulations of clock genes have the possibility to disrupt the rhythms of these genes and to elicit inhibitory effects on cell proliferation or hormonal excretion. Further study to examine the effect of Cd on circadian rhythms of these clock genes will provide more reliable results.

VARIANT GLUTATHIONE S-TRANSFERASE P1 PROTEIN EXHIBITS DIFFERENTIAL ENZYMATIC ACTIVITY AND MERCURY INHIBITION.

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The effect of chronic exposure to the potent neurotoxicant, mercury (Hg), on the human body is influenced by genetic polymorphisms in glutathione s-transferases (GST). GSTs mediate the metabolism and elimination of Hg. While polymorphic GSTs have been linked to altered body burden of mercury in humans, the mechanisms underlying these associations remain elusive. Here we utilize two in vitro methods (enzyme biochemistry and bacterial growth) to characterize the effects of one common polymorphism in GST pi 1 (GSTP1: Ile105Val) in the presence of mercury. GSTP1 105Ile and GSTP1 105Val were expressed by the proteins in E. coli in Escherichia coli. The enzymatic activities of the proteins were compared in the presence and absence of inorganic (HgCl2) and organic mercury (MeHgCl). Enzymatic activity assays performed with substrates glutathione and 1-chloro-2,4-dinitrobenzene revealed that the two versions to have differing kinetic parameters. Inhibition assay with MeHgCl suggest that GSTP1 105Val (IC50=55.2 ±15μM) is more sensitive to inhibition than 105Ile (IC50=30.5±11μM). The two enzymes had similar IC50s for HgCl2 (19.7 vs. 15.7 μM for 105Ile and 105Val, respectively). Compared to MeHgCl, HgCl2 is a more potent inhibitor of GSTP1 activity. The cytoprotective role of GSTP1 105Ile and 105Val was assessed by expressing the proteins in E. coli treated with HgCl2 and comparing the growth rates with a control. In this model, GSTP1 expression did not improve cell survivability. In conclusion, our use of two in vitro methods that the GSTP1 105 polymorphism differs between enzymatic activity in the presence of mercury, and this may affect the toxicokinetics of Hg in the body. When these in vitro results are coupled with epidemiological evidence, such gene-environment studies may help identify susceptible subpopulations, improve our understanding of the mechanisms underlying Hg neurotoxicity, and minimize variation associated with exposure biomarkers commonly used in risk assessment.
Mercury (Hg) is a potent neurotoxin of concern to both the general public and occupationally exposed workers (e.g. dentists). Recent studies suggest that several genes mediating the toxicokinetics and toxicodynamics of Hg are polymorphic in humans. This work hypothesizes that single nucleotide polymorphisms (SNPs) in key glutathione, metallothionein, and selenoprotein genes underlie inter-individual differences in Hg body burden as assessed by analytical biomarker analysis of urine and hair. A population of 232 dental professionals was recruited during the 153rd Michigan Dental Association (MDA) Annual Convention in Lansing, MI. Samples of urine (biomarker for inorganic Hg) and hair (organic Hg) were collected, and total Hg content was measured. Average urine (1.1±1.2 μg/L) and hair mercury levels (0.54±0.7 μg/g) were similar to national averages from the National Health and Nutrition Examination Survey (NHANES). Information on dietary fish consumption and potential confounders were obtained from questionnaires. Taqman assays were used to genotype DNA from buccal swab samples at 20 polymorphic sites in genes implicated in Hg metabolism (e.g. GCLC-129, SEPP1-234, MT1A-2835) expressed genes in the sub-, low- and high toxic MeHg exposures and 44, 424 and 876 differentially expressed genes in the sub-, low- and high toxic HgCl2 exposures and 44, 424 and 876 differentially expressed genes in the sub-, low- and high toxic HgCl2 exposures. Analysis of the microarray data using principal components analysis, hierarchical clustering, and self-organizing maps indicated that C. elegans have different transcriptional responses when exposed to different mercury species. 599 genes up-regulated by one or both mercurials (258 HgCl2, 276 MeHg, 65 both) in the microarray study were selected for search for genes involved in mercurial resistance. Mercurial resistance genes were identified by using RNAi to knock down gene expression then separately exposing nematodes to 10 μM HgCl2 and 3 μM MeHg. Mercurial resistance genes were defined as those in which co-exposure to mercury and double-stranded RNA resulted in a synergistic decrease in growth. We identified 163 genes (65 HgCl2 only, 45 MeHg only, 53 both mercurials) that were important in resistance to mercury. C. elegans appear to have both unique and overlapping mercurial resistance mechanisms.
involved in the enhanced production of ROS in response to MeHg through an as-yet unknown mechanism that is independent of the activity of electron transport system complex III.

Thus, Rip1 is potentially involved in the enhanced production of ROS in response to MeHg through an as-yet unknown mechanism that is independent of the activity of electron transport system complex III.

Association between the risk of cardiovascular diseases (CVDs) and mercury exposure has been frequently described, yet the mechanism underlying remains poorly understood. We investigated the effects of low-level of mercury on erythrocytes in an effort to explore the roles of erythrocytes in mercury-induced CVDs. In freshly isolated human erythrocytes, prolonged exposure to low dose Hg²⁺ induced remarkable shape changes from discocytes to echinocytes and further into spherocytes as observed by SEM. During these shape changes, microvesicles(MV) were generated as determined by FACs and confocal microscopy analysis. These MV and remnant erythrocytes were shown to express phosphatidylserine(PS), important mediator for procoagulant activation. Hg²⁺-induced MV generation and PS exposure was mediated by the concomitant inhibition of flipase, an enzyme recovering PS into inner leaflet, and activation of scramblase, an enzyme scrambling lipid asymmetry. Intracellular calcium increase, which is a major factor for these enzyme activity changes, was increased significantly by Hg²⁺. In addition, Hg²⁺ treatment induced significant depletion of ATP and protein thiol, critical factors for flipase inhibition and scramblase activation. Thioli supplement reversed Hg²⁺-induced MV generation and PS exposure along with Ca²⁺ increase and ATP depletion, indicating that free-thiol depletion was a key mediator for these events. In line with these increases in PS exposure and MV generation, Hg²⁺-treated erythrocytes accelerated thrombin generation and adhered to HUVEC efficiently. These Hg²⁺-mediated procoagulant activation of erythrocytes in vitro could be further confirmed in vivo rat venous thrombosis model, where Hg²⁺ administration increased thrombus formation significantly. In conclusion, our study demonstrated that mercury exposure could provoke procoagulant activity in erythrocytes through protein-thiol depletion mediated PS exposure and MV generation, contributing to enhanced thrombus formation.
1840 LEAD AFFECTED UPTAKE OF GLUTAMATE IN GLIAL PLASMALEMMAL VESICLES AND PRE-SYNAPTIC NERVE TERMINALS.
Glial Plasmalemmal Vesicles are a sample of the plasma membrane pinched off from the glial origin representing the Astrocytic sample. Synaptosomes are pre-synaptic nerve terminals. Both play a vital role in regulating extracellular Glutamate concentrations. The study is designed to ascertain the effect of Lead on glutamate uptake and the involvement of secondary factors in the mechanism of lead affected glutamate uptake. Glial Plasmalemmal Vesicles and Synaptosomes are isolated using Percoll Density Gradient centrifugation of the rat hippocampal homogenate. Glutamate uptake is found to be linear for two minutes as seen by the time-dependent studies done with L-[3H]-Glutamic acid. This uptake is found to be sodium-dependent. On treatment with excitatory amino acid transporter 2 inhibitor Dihydrokainate(100μM), a decrease in glutamate uptake for GPV and synaptosomes is observed. Lead poisoning is one of the most common diseases in children impacting glutamate concentrations and hence the effects of lead (1mM) on glutamate uptake in both the systems are studied. It shows a 4x to 5x fold increase in glutamate uptake in Glial Plasmalemmal Vesicles and synaptosomes. This lead affected glutamate uptake is found to be protein kinase C and transporter dependent, as seen by inhibition in lead affected uptake by the Protein Kinase C inhibitor and Excitatory amino acid transporter 2 inhibitor. The MTT assay protocol ensures the fractions are viable in the time frame of the experiment. The assay also shows that lead treatments do not have detrimental effect on glial plasmalemmal vesicle and synaptosomes.

1841 ACTIVATION OF PROTEIN KINASE C IN LEAD-INDUCED ACCUMULATION OF β-AMYLLOID IN THE CHOROID PLEXUS: RELATIONSHIP TO SUBCELLULAR RELOCATION OF LOW DENSITY LIPOPROTEIN RECEPTOR PROTEIN-1 (LRP1).
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The choroid plexus (CP), a brain tissue constituting the blood–cerebrospinal fluid barrier, has the capacity to remove beta-amyloid (Aβ) from the cerebrospinal fluid (CSF). Our previous work indicates that exposure to lead (Pb) results in Aβ accumulation in the CP by decreasing the expression of low density lipoprotein receptor protein-1 (LRP1), a protein involved in the transport and clearance of Aβ. The current study was designed to explore the relationship between Aβ accumulation, protein kinase C (PKC) activity, and LRP1 status in the CP following Pb exposure. Results from confocal microscopy revealed that LRP1 was primarily localized in the cytosol of the CP in control rats and migrated distinctly to the apical surface and the microvilli with acute Pb exposure (27 mg Pb/kg, ip, 24 hr). Co-immunostaining revealed a co-localization of both PKC-δ and LRP1 in the cytosol of control rats, with a distinct re-localization of both towards the apical membrane following Pb exposure. Pre-incubation of the tissues with PbC-δ inhibitor rottlerin (2 μM) prior to Pb exposure, in vitro, abolished the Pb-induced re-localization of LRP1 to the apical surface. Importantly, a significant elevation in intracellular Aβ was observed in the cytosol of the CP following Pb exposure, which was abolished following preincubation with rottlerin. Interestingly, treatment with rottlerin alone in controls also caused a re-localization of Aβ from the cytosol to the nucleus in CP tissues. Finally, co-immunoprecipitation studies revealed a strong protein-protein interaction between LRP1 and PKC-δ in the CP. These studies suggest that Pb exposure disrupts Aβ homoeostasis at the CP, owing partly to a Pb-induced re-localization of LRP1 via activation of PKC-δ. (Supported in part by NIH/NIEHS ES-008146 and ES-017055)

1842 LONG TERM TREATMENT OF LEAD POISONING AFTER RETAINED BULLET WITH DIMERCAPTOSUCCINIC ACID.
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Our objective is to present a case of symptomatic lead toxicity with severe anemia, symptomatic impairment of peripheral and central nervous systems (paresthesia, insomnia, trembling hands) following a gunshot wound, 24 years previously. The patient had 235 μg/dL of Pb in blood (PbB) concentrations. Initial treatment consisted in dimercaptosuccinic acid (DMSA) followed by surgery. The main outcome was the reduction of PbB levels with improvement of symptoms. Because the current rise in PbB levels, the patient has been treated with a monthly scheme of five days with DMSA (500 mg/12 h) during four years. We discussed that lead residues of the bullet and a large body burden of lead were the main causes for presenting persistent high PbB concentrations. This is the first time of long term therapeutic scheme of DMSA to control PbB levels without adverse effects. The results support to encourage a prompt surgical extraction of retained bullets to avoid lead dispersion around the bullet.

1843 CHARACTERIZATION OF GENE EXPRESSION ALTERATIONS INDUCED BY DEVELOPMENTAL EXPOSURE OF ZEBRAFISH TO LEAD (Pb).
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Despite its removal from gasoline and paint decades ago, the adverse effects of Pb exposure is still a critical issue. In developing children, Pb exposure has been linked to reductions in IQ and increased incidence of behavioral disorders. Furthermore, a growing number of reports suggest that adverse effects to children might occur well below the current CDC mandated action level of 10 μg/dL. While the toxicity of Pb has been extensively studied, the specific mechanisms of toxicity which are responsible for the deficits observed in the developing neurological system at these lower concentrations have not been elucidated. It is, therefore, of utmost importance to further study the effects of Pb in a developing organism at these low relevant exposure concentrations. To begin this investigation, zebrafish embryos were exposed to 10 μg/dL Pb, starting shortly after fertilization. Comparative gene expression analysis was performed using a high-density oligo microarray at 3 and 5 days post-fertilization (dpf). Gene lists, comprised of genes that were at least 1.5 fold up or down regulated in all replicates at that specific time point, were generated. At 3 dpf, 77 genes were differentially expressed with the majority of genes (70) being down-regulated. Of particular interest, were a large number of genes involved in neuronal navigation and neuronal development, providing possible evidence for novel mechanisms of developmental toxicity. Other gene categories with significant decreases in expression included collagen and cell structure related genes. At 5 dpf, a different expression pattern was observed with 170 genes up-regulated and 144 genes down-regulated in Pb-treated samples compared to the controls. Interestingly, many genes down-regulated at 3 dpf were up-regulated at 5 dpf. These results are possibly due to delayed developmental processes or compensatory mechanisms. Further work will focus on the characterization of the Pb-induced changes observed in previously unidentified genetic pathways and the mechanisms and functional consequences of these alterations.

1844 CORRELATION BETWEEN LEAD LEVELS IN HAIR AND SOME BOVINE TISSUES.
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The goal of the present investigation was to establish the role of lead in hair as a reliable indicator for the determination of lead in tissues of the exposed animals. Thus, lead levels in hair can serve as an early warning sign. A total number of 50 male cattle and 50 male buffaloes were subjected to this investigation. Whole blood, hair, liver, kidney, muscle, and bone samples were collected from each animal and used for the determination of lead. The recorded results were as follows:

(A) The concentration of lead in examined tissues for cattle showed lead concentration values (X ± SE) 0.342 ± 0.055, 0.039 ± 0.003, 0.211 ± 0.033, 0.220 ± 0.015, 0.11 ± 0.013 and 0.178 ± 0.016 (ppm) in the hair, blood, liver, kidney, muscle, and bone, respectively. While results in buffaloes showed lead concentration values (X ± SE) 0.147 ± 0.018, 0.023 ± 0.0032, 0.061 ± 0.011, 0.131 ± 0.014, 0.032 ± 0.0026 and 0.072 ± 0.010 (ppm) in hair, blood, liver, kidney, muscle, and bone, respectively. (B) Correlations between lead concentrations in hair and other investigated tissues in cattle revealed significant positive correlations between levels in hair and blood (p < 0.05); hair and muscle (p < 0.01). On the other hand, buffaloes showed significant positive correlations between lead concentrations in hair and blood (p <0.01), and hair and bone (p <0.01).
1845 SYSTEMATIC SCREENING OF YEAST SUSCEPTIBILITY GENES TO CADMIUM, LEAD AND ZINC TOXICITY.

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The metals cadmium (Cd), lead (Pb) and zinc (Zn) are commonly found as contaminants in the environment. Yeast mutant strains, containing single deletions for almost every gene in the yeast genome, can be assayed in parallel to identify susceptibility genes to metal toxicity in yeast. Using a microarray-based method and measuring growth as an endpoint, we determined how the individual genotype of each deletion strain impacts their fitness in the presence of these toxicants. Based on the high degree of conservation between human and yeast genes, we used this approach to screen gene-environment interactions in yeast with potential relevance to humans, as we have previously shown for arsenic. The genetic requirements for yeast’s resistance to Cd, Pb and Zn included both common and specific genes. Treatments with these three metals identified genes mainly associated with highly-conserved intracellular transport pathways. Another category included genes related to maintenance of metal homeostasis, especially of iron. Of particular interest were metal-specific resistance genes. Cd resistance-specific genes included GSH1, GRO3 and ROK1 provided evidence of the involvement of oxidative stress. The genes FUC2 and VPH1 were essential only in Zn, while ARE1 in Pb. Of note, is that many of these yeast genes have human orthologs, which may play a role in the cellular response to metal toxicity in the same manner as their yeast counterparts. In contrast, deletion of the genes BST1, MRPL11, YGR272C and ZAP1 resulted in increased fitness of the corresponding deletion strains, indicating that their presence is detrimental for yeast under toxic concentrations of Cd, Pb and/or Zn. In conclusion, the parallel analysis of yeast mutants identified novel susceptibility genes to Cd, Pb and Zn toxicity.

1846 INVESTIGATION OF TECHNIQUES TO MAXIMIZE THE POTENTIAL OF DRIED BLOOD SPOT ANALYSIS (DBS) TO REDUCE SAMPLE VOLUMES IN MOUSE TOXICOLOGIC STUDIES.


Toxicokinetic investigations supporting non-clinical studies traditionally involve bioanalysis of plasma samples. Advances in technology, however, mean that use of Dried Blood Spot (DBS) samples is now being widely proposed. For this analysis whole blood is spotted onto DBS cards, and allowed to dry at room temperature prior to storage and/or shipment for analysis. The number of mice required for toxicokinetic analysis is governed by the size of the animals, the blood volume needed and the number of time-points being investigated. Use of the DBS analysis can substantially reduce the size of the sample required and hence allow a greater number of samples to be collected from one animal thereby reducing the number of animals on study. Previous publications relating to DBS analysis have not focussed on maximising the potential for reduced blood volume, which has particular value in mouse and also rat studies. In Phase 1 of this study blood samples were collected from the tail vein of mice, previously dosed with an investigative drug, using four different techniques. DBS samples were prepared and analysed. Assessment of the data established the preferred method of sampling. In Phase 2 of the study, mice were similarly dosed and samples of whole blood collected at 6 time-points after dosing. DBS samples were prepared and analysed using a protein precipitation and LC-MS/MS validated method. For comparison, blood taken at the same time-points was sampled into conventional tubes and was also analysed, similar results confirmed the validity of the DBS method. This study demonstrated the preferred method of collecting whole blood from mice for DBS analysis and confirmed that reliable results for toxicokinetic analysis can be obtained using these techniques from fewer mice during regulatory preclinical studies.


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Since the initial findings in 2002 by researchers at the Swedish National Food Administration and Stockholm University of the presence of acrylamide (AA) in a variety of fried and oven-baked foods and its formation is associated with traditional high temperature cooking of certain carbohydrate-rich foods, similar findings have been reported elsewhere, including the US. As a potential human carcinogen and a known human neurotoxicant, AA presence in foods may pose a public health concern. In the National Health and Nutrition Examination Survey (NHANES 2003-04) hemoglobin adducts of acrylamide (AA-Hb) and its metabolite glycidamide (GLY-Hb) were measured in over 7,000 NHANES participants age 3 year and older. Dietary intake information from 24-hr dietary recalls and a food frequency (FFQ), lifestyle data (smoking and blood cotinine), demographic data (age, gender, ethnicity) and anthropometric measurements (body weight, BMI, etc.) are also available from NHANES. Using a Monte Carlo method, the 24-hr dietary recall and FFQ data were combined with AA concentration data from US FDA to estimate long-term usual dietary AA exposure. The correlations between usual dietary AA exposure and AA-Hb and GLY-Hb were evaluated using simple and multivariate linear regressions (MLR). Smoking (defined by blood cotinine), age, gender, energy and macronutrient intake, BMI, and activity level were included as covariates in the MLR models. The results showed that while dietary AA sources positively correlate with AA-Hb and GLY-Hb (p < 0.05), the magnitude of the correlation is quite small (R-Squared < 3%). Also, relative to the background adduct levels observed in the NHANES population the incremental increase in AA-Hb and GLY-Hb from dietary AA sources is small. In light of this finding that dietary sources of AA are not a particular concern for AA-Hb and GLY-Hb, non-diaryt sources of AA exposure should be examined in future research.

1848 SECOND ORDER RATE CONSTANTS FOR THE IN VITRO REACTION OF THE TOXIC INDUSTRIAL CHEMICAL ACRYLONITRILE WITH THE MOST REACTIVE SITES IN HUMAN BLOOD.

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The twenty-first century warfighter and civilians will encounter the challenge of the potential use of toxic industrial chemicals (TICs) as chemical warfare agents. Preparedness for acts of chemical terrorism and warfare require prevention, detection and response to such attacks. Appropriate chemical testing will allow for proper diagnosis and treatment of exposed individuals. Acrylonitrile (AN) is a TIC produced in large quantities by the chemical industry and is acutely toxic. Our overall objective is to define the chemical signatures of AN-adducts in human blood, and to eventually devise a rapid, high throughput screening technology to enable examination of large groups of individuals following a known or suspected exposure. Toward this end, in this project we have measured the second order rate constants for the reaction of AN with the most reactive sites in human blood in vitro. Fresh human blood, red blood cell lysates and plasma were incubated, under pseudo first-order conditions, with 100 mM AN at 37°C and the disappearance of glutathione (GSH) and the appearance of the AN-adducts of GSH, hemoglobin β-Cys93 (HbβC93-AN) and albumin Cys34 (AbC34-AN) were monitored by spectrophotometry (spec) and/or high resolution mass spectrometry (MS). The second order rate constants in M⁻¹s⁻¹ were as follows: disappearance of GSH in whole blood, 0.0813 (spec) and 0.0799 (MS); appearance of GS-AN in whole blood, 0.0776 (MS), appearance of HbβC93-AN in rbc lysate, 0.00722 (MS) and appearance of AbC34-AN in plasma, 0.224 (MS). The data indicate that the most reactive sites for AN in human blood are Cys93 of α and β globin chains. This site reacts 2.8 times faster than GSH and 310 times faster than HbβC93. Future studies will be directed toward determining the limits of detection of this adduct and minimizing the analysis time. Supported by DOD, U.S. Army Medical Research and Material Command, W81XWH-08-1-0047.

1849 DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF BISPHENOL A AT TRACE LEVELS IN HUMAN BLOOD AND URINE AND ELUCIDATION OF FACTORS INFLUENCING METHOD ACCURACY AND SENSITIVITY.

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Bisphenol A (BPA) is an industrial chemical used to make polymers including some used in food contact applications. Virtually complete presystemic clearance of orally administered BPA occurs in humans by metabolism to BPA-glucuronide (BPA-G), but some biomonitoring studies report low concentrations of free (parent) BPA in human blood and urine. Trace contamination of BPA from exogenous sources or hydrolysis of BPA-G to free BPA, either during or after biomonitoring specimen collection, may have contributed to the reported concentrations of free BPA. An analytical method for the determination of free BPA in human blood and
urine was developed and validated in two independent laboratories, using the latest generation of HPLC/MS/MS instrumentation to ensure the desired high sensitivity and selectivity. The method was designed to account for and/or eliminate background contamination from all sources, and operated by multiple parties (the method could occur from devices used for specimen collection or storage, as well as other sources. The method employed an internal standard (28BPA) and demonstrated accuracy and reproducibility in both matrices fortified with BPA or a surrogate analyte (13C-BPA) at a low quantitation limit (0.1 - 0.2 ng/mL). For validation, five replicate samples were analyzed to evaluate accuracy and reproducibility. Importantly, it was demonstrated that the conditions of the method did not result in the hydrolysis of BPA-G to free BPA, another possible source of error in BPA analysis. Application of the principles defined by this method will be critical to assure valid analytical results in any future biomonitoring studies.

1850 ASSESSMENT OF ENVIRONMENTAL DATA COLLECTED IN A COMMUNITY WITH NUMEROUS PETROLEUM REFINING AND PETROCHEMICAL FACILITIES.

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Corpus Christi, Texas is the 6th largest port in the US (by tonnage shipped), and home to a number of petroleum refining and petrochemical facilities. The area has one of the most dense and rigorous air toxicity monitoring networks in the US – 18 monitors blanketing the industrial corridor; multiple types of monitors (auto-GCs and canister based); co-locating monitors; monitors operated by multiple agencies (the state, a university, and industry); and an extensive period of record. As a result, there is a tremendous amount of actual data available to assess potential health impacts. Our analyses focused on benzene, the only chemical to be measured above state guidelines, and involved the following: development of statistical measures of short- and long-term concentrations, assessment of trends, and evaluation of potential impacts on human health. Annual average benzene concentrations have steadily declined over time and have all been below the long-term state health-based guideline (HBG) (1.4 ppbv) since 2006. Of the over 99,000 hourly samples collected at the 3 auto-GCs, there were only 3 that slightly exceeded the short-term state HBG (180 ppbv). Air monitoring data were also used to facilitate interpretation of benzene biomonitoring data collected in the community. A regression analysis was conducted to develop a relationship between ambient air and blood concentrations based on data in the published literature. Air concentrations of benzene predicted based on this analysis were then compared to concentrations measured at monitors near the study neighborhood. The maximum monitored air concentration measured during the study period (73 ppbv) was over 34 times lower than the predicted air concentration associated with the median blood benzene measurement. A principal component analysis (PCA) of BTEx (benzene, toluene, ethylbenzene, and xylene) in blood and air showed that these media had fundamentally different fingerprints. Taken together, these findings suggest that the benzene levels in blood are not likely related to ambient air.

1851 JOB-BASED ANALYSIS OF BENZENE AIR CONCENTRATIONS ASSOCIATED WITH REFINERY OPERATIONS.

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In this study, historical exposures to benzene were quantified for workers at four U.S. refineries from 1976 to 2006. The results of more than 12,000 long-term (≥ 180 minutes) personal industrial hygiene air samples were evaluated in this analysis. The dataset represents a targeted air sampling approach and the benzene detection frequency was approximately 43%. Overall, the results indicate that long-term personal exposures were generally below the OSHA occupational exposure limit (OEL) of 1 ppm over the past 30 years. The arithmetic mean and 95th percentile for all workers at these refineries was approximately 0.21 and 0.44 ppm, respectively. In addition, average air concentrations of benzene were statistically different by operational status (routine, turnaround, startup), and thus all subsequent analyses were conducted on the dataset divided accordingly. The jobs most frequently sampled included those with potential contact with refinery product streams including process technician, machinist, pipetter/welder, and laboratory technician. The ANOVA indicated that for most jobs, the associated air concentrations associated were not dependent on refinery area. However, certain areas, such as waste treatment, reformer, tank farm, and lube extraction unit, were found to be statistically different from other areas of the refinery for several different job titles; and therefore, specific job categories were created. Changes over time in benzene air concentrations were also evaluated for these job categories. For several job categories, there was a statistically significant decrease in benzene air concentration between the 1976-1989 period and 1990-2006 periods. This study provides a focused analysis of occupational exposure to benzene during refinery operations, and it should be useful for estimating exposures to benzene for refineries over the past 30 years.

1852 USING THE BIOMONITORING EQUIVALENT FOR BENZENE TO HELP INTERPRET BIOMONITORING DATA.

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A Biomonitoring Equivalent (BE) is the concentration or range of concentrations of a biomarker of exposure for a chemical consistent with existing exposure guidance values. Substantial efforts have been developed over the past two decades to biomonitor for benzene exposures in a range of occupational (primarily) and environmental (more recently) exposure scenarios. Biomonitoring for occupational exposures poses fewer problems for interpretation because exposures are usually elevated over environmental (background) exposures and the timing of exposures are known and the timing of sample collection can be controlled. Biomonitoring for environmental exposures poses more challenges because exposures are often erratic and the contamination at the sample collection site can be variable. BEs provide a means of interpreting population-based biomonitoring studies for environmental exposures in a public health risk context.

1853 CLINICAL METHOD TO ASSESS SITE OF USE EXPOSURE TO SMOKELESS TOBACCO CONSTITUENTS.

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The use of smokeless tobacco (ST) products at the same oral site may result in an acute injury characterized by ulceration and inflammation that is reversible upon elimination of same site use. Yet the mechanism and constituents responsible are not clear. Some studies have measured constituent levels in spit or whole saliva during product use, but not at the site where the injury occurs. This study determined the site of tobacco placement using two saliva collection techniques. Saliva samples were collected in nine adult ST users through spitting and either by aspirating (AS) saliva from the ST quid at different time points up to 35 minutes or by removing and centrifuging (CF) the quid at the end of each session (5, 15, or 25 min). The average pH, osmolality and nicotine levels were measured on whole saliva samples. The methodology was performed over 14 separate days for each subject. Overall, the results indicate that there is a tremendous amount of actual data available to assess potential health impacts. Annual average benzene concentrations have steadily declined over time and have all been below the long-term state health-based guideline (HBG) (1.4 ppbv) since 2006. Of the over 99,000 hourly samples collected at the 3 auto-GCs, there were only 3 that slightly exceeded the short-term state HBG (180 ppbv). Air monitoring data were also used to facilitate interpretation of benzene biomonitoring data collected in the community. A regression analysis was conducted to develop a relationship between ambient air and blood concentrations based on data in the published literature. Air concentrations of benzene predicted based on this analysis were then compared to concentrations measured at monitors near the study neighborhood. The maximum monitored air concentration measured during the study period (73 ppbv) was over 34 times lower than the predicted air concentration associated with the median blood benzene measurement. A principal component analysis (PCA) of BTEx (benzene, toluene, ethylbenzene, and xylene) in blood and air showed that these media had fundamentally different fingerprints. Taken together, these findings suggest that the benzene levels in blood are not likely related to ambient air.

1854 BAYESIAN FEATURE SELECTION IDENTIFIES HUMAN PLASMA PROTEOMIC BIOSIGNS OF SYSTEMIC CHRONIC INFLAMMATORY AND OXIDATIVE STRESS.

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Exposure to cigarette smoke and obesity are two of the most important risk factors for human morbidity and mortality. Increasingly, there is evidence that the oxidative stress resulting from chronic inflammatory stimuli is the unifying mechanism...
underlying the development of co-morbidities. Thus, identifying protein biomarkers of oxidative stress in human populations could give important insight to understand the development and progression of complex disease by accurately and quantitatively assessing the individual’s exposure to environmental stressors and their individual’s responses to these stressors. We compared a subset of plasma samples from representative participants in a cohort of 500 exposed to mainstream tobacco smoke or never-smoked with BMI above 35 or below 25. There were 27 individuals selected from the four representative groups: 1/6 high BMI non-smoker, 2/7 high BMI smoker, 3/7 low BMI non-smoker, and 4/7 low BMI smoker. Each sample was analyzed using high mass accuracy LTQ-Orbitrap-MS and a total of 6459 peptides were identified with statistical confidence in at least 1 of the 27 samples. Statistical analyses found 1958 potential biomarkers. The goal of this study was to derive biomarkers of response and to evaluate screening potential. We developed a Bayesian feature selection approach that uses supervised learning, specifically partial least squares discriminant analysis, in a cross-validated manner to iteratively identify the most probable peptide biomarkers. Features are added sequentially to the model to optimize the posterior probability of a correct classification across all samples. We find that with as few as 13 peptides over 90% of the samples can be correctly classified into the four key BMI/Smoking combinations. Only 2 peptide biomarkers are required to simply classify BMI and smoking status, respectively. Supported ES 016015.

1855 ACTIVATED CHARCOAL FILTERS PROVIDE LITTLE PROTECTION FROM ETS GENERATED CO AND TSP EXPOSURE AND LUNG INFLAMMATION.

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Exposure to high levels of environmental tobacco smoke (ETS) remains a serious problem for many workers. Seeking exemptions to smoking bans, some industries claim that air filtration systems provide adequate protection to workers from ETS, although these claims have little scientific basis. In this study we tested whether an air filtration system (activated charcoal filter) could significantly reduce ETS contaminants and provide adequate protection against ETS induced inflammatory response in the lungs. Using a team of 10 smoking machine to generate SHS as a surrogate for ETS, we exposed baboons to ETS for 6 hours a day, 5 days a week for 6 weeks. Mice were exposed to either full ETS, ETS passed through an activated charcoal filter or no ETS. Carbon monoxide (CO) and total suspended particle (TSP) analyses showed that the filters reduced CO and TSP by 32% and 35% respectively. Bronchoalveolar lavage (BAL) fluid collected to assess inflammatory cell infiltration into the lungs by flow cytometry showed that full ETS exposure resulted in an increased infiltration of macrophages into the BAL fluid of the exposed mice as compared to the non-exposed mice and that passing the ETS through an activated charcoal filter did not eliminate the macrophage infiltration. Granulocyte levels were highest in the BAL fluid of the animals receiving the filtered ETS followed by full ETS and then the unexposed animals. These results demonstrate that activated charcoal filters do not eliminate exposure to ETS chemical constituents and suggest that the animals exposed to the filtered ETS may be at a different stage in their inflammatory response but are not fully protected from the ETS induced inflammatory response. This study was supported by a grant from the Flight Research Laboratory, U.S. EPA, Research Triangle Park, NC, 2National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC, 3Biostatistics Program, Department of Statistics, North Carolina State University, Raleigh, NC and 4National Center for Environmental Assessment, U.S. EPA, Research Triangle Park, NC.

Numerous air pollution epidemiologic studies have observed associations between ambient concentrations of particulate matter (PM) and increased rates of morbidity and mortality. These studies often use measurements from central-site ambient monitors as exposure surrogates. To better understand the linkages between ambient concentrations, exposures, and adverse effects in diabetics and asthmatics, we are developing an air pollution exposure model for individuals (EMI) in health studies. The EMI predicts personal exposures from ambient concentrations and questionnaire information such as indoor sources and time-activity patterns. A critical aspect of the EMI is estimation of PM concentrations within homes where people spend most of their time. We developed a mass-balance residential indoor air quality model to predict daily indoor PM10 mass concentrations from outdoor concentrations and questionnaires. The air exchange rate (AER), a critical model parameter, was estimated with a mechanistic AER model. Other parameters were set to reported literature values. The model was evaluated with data from the Research Triangle Park (RTP) Particulate Matter Panel Study, which measured daily personal, residential indoor and outdoor, and ambient PM10 mass concentrations for seven consecutive days during each of four seasons in 36 homes within the RTP area of North Carolina. For the model-predicted and measured indoor concentrations of ambient-generated PM10 mass, the median absolute difference was 13.7% (1.4 μg/m3). Our study demonstrates the feasibility of using the model to predict indoor PM10 concentrations in support of developing exposure-dose metrics for health studies. This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect Agency policy.

1857 ALTERATION OF PERIPHERAL BLOOD MONOCYTE GENE EXPRESSION IN HUMANS FOLLOWING DIESEL EXHAUST INHALATION.

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Epidemiologic associations between increased cardiorespiratory morbidity and mortality and particulate air pollution are well-established and most strongly correlated to particulate air pollution. In order to identify potential mechanisms, the response of peripheral blood mononuclear cells (PMBC). Key responders to combustion derived particulate matter, to fresh diesel exhaust was used as a model for particulate air pollution. Out of a bank of 100 healthy non-smoker subjects, 14 ethnically homogeneous (white males) and normally age-matched (21–30) subjects were selected for analysis. All participants gave written informed consent approved by the UMDNJ IRB. Sixty minute seated inhalation exposures were conducted on 2 separate days with clean air or freshly generated and diluted diesel exhaust at a concentration of 300μg/m3 PM2.5 in the Controlled Exposure Facility located within EOHSL. Prior to and 24 hrs following each session, whole blood was sampled and fractionated. PMBC collection, RNA extraction, and subsequent generation of cDNA were followed by hybridization with Agilent Whole Human Genome(4X44K) arrays. Three pathways, oxidative stress, the ubiquitin proteasome pathway, and the coagulation system, were identified by analysis of differentially expressed genes using Ingenuity Pathway Analysis software. Nine genes from these altered pathways were validated using real-time PCR to compare fold change in expression between diesel exposed and control days. Quantitative gene fold changes generated by real-time PCR were consistent with the directional fold changes from the microarray analysis. The largest fold changes were observed in genes from the coagulation pathway, namely F2R(+30.8) and PLAU(-25.2). Changes in gene expression connected with key pathways are likely to underlie observed physiologic and clinical outcomes, such as increased clot formation and reduced vascular responsiveness. Correlations between gene transcriptional changes with air pollution exposure and their observable physiologic counterparts will be discussed.

1858 TOXICITY EFFECT OF DIESEL EXHAUST PARTICLES ON BIOMECHANICS AND CELL SURFACE BIOPOLYMERS OF HUMAN LUNG CARCINOMA EPITHELIAL CELLS A549: PRELIMINARY STUDY BY ATOMIC FORCE MICROSCOPY/RAMAN MICROSCOPY.

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Toxicological and epidemiological studies have indicated that air pollution, or particulate matter (PM), is an environmental health risk factor for respiratory disorders and diseases. Although cytotoxicity and genotoxicity of human lung cells associated with diesel exhaust particles (DEP) exposure have been well documented, the underlying biomechanics role in such cell damage due to DEP interaction not fully understood. Current in vitro evaluation techniques of DEP toxicity are limited, invasive, and slow. In this study, we applied atomic force microscopy (AFM) Raman spectroscopy to in vitro measure the alterations of bio-mechanical and nanoarchitectural properties and cell biopolymers of living human lung carcinoma epithelial A549 cells exposed to DEP over time. AFM indicated that DEP treatments induced the variations in membrane surface hydrophobicity: the significant decreases in membrane surface adhesion force over interaction time, despite cell elasticity did...
not alter significantly at our experimental conditions. Raman spectroscopy analysis, as a non-invasive technique, revealed the changes in cell surface biocomponents according to the changes of the intensities of their characteristic bands: 785 cm⁻¹ (DNA), 959 cm⁻¹ (carbohydrates), 1086 cm⁻¹ (proteins), and 1092 cm⁻¹ (lipids) for treated and untreated cells. In addition, fluorescence microscopy images showed the changes of cytoskeleton structure due to DEP interaction. These findings suggest DEP triggers important biological mechanism that is closely interrelated with biochemical, bio-mechanical, and cellular architectural changes in the exposed human lung cells.

1859 TOXICITY OF LUNAR DUST IN LUNGS ASSESSED BY EXAMINING BIOMARKERS IN EXPOSED MICE.

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NASA plans to build an outpost on the Moon for prolonged human habitation and research. The lunar surface is covered by a layer of fine soil, of which the finest portion is highly reactive dust. Because the toxicity of lunar dust is not known, its toxicity in the lungs of exposed mice was investigated in the present study. Dust samples of respirable sizes were aerodynamically isolated from two lunar soil samples of different maturities (cosmic exposure ages) collected during the Apollo 16 mission. The lunar dust samples, titanium dioxide, or quartz (Min-U-Sil-5), suspended in normal saline, were given to groups of 5 mice (C-57 male) by intrapharyngeal aspiration at 1, 0.3, or 0.1 mg/mouse. The mice were euthanized 7 or 30 days later, and their lungs were lavaged to assess the presence of toxicity biomarkers in bronchoalveolar lavage fluids. The acellular fractions were assayed for total proteins, lactate dehydrogenase activities, and cytokines; the cellular portions were assessed for total cell counts and cell differentials. The overall results showed that lunar dust was more toxic than titanium dioxide, but less toxic than quartz. The two lunar dust samples showed similar toxicity.

1860 DNA DAMAGE DETECTED BY MICRONUCLEI AND COMET ASSAYS IN LYMPHOCYTES ISOLATED FROM WORKERS EXPOSED TO COKE OVEN EMISSIONS.

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Objective: To investigate the genotoxic effects of coke-oven emissions in workers at different operating locations. Methods: a total of 304 exposed workers were recruited, including 186 subjects working either at the top or side of the coke-oven (high exposure group) and 118 workers with job locations at the bottom of oven (low exposure group). In addition, 56 control subjects were recruited by frequency match with those exposed workers by age and self-reported cigarette smoking. The air concentrations of benzo(a)pyrene (BaP) were monitored for 3 consecutive days at the top, side, and bottom of coke-oven, respectively. Micronuclei (MN) and comet assays were conducted in peripheral lymphocytes isolated from all participating subjects. Results: BaP concentrations monitored at top, side, and bottom of coke-oven were 0.27±0.05, 0.13±0.03, and 0.04±0.01 pg/m³, respectively. The demographic data showed no significant difference in age and smoking status among control, high and low exposed subjects. However, significant higher frequencies of MN were observed in high (2.89‰) and low (2.04‰) exposed groups as compared with controls (1.45‰, p<0.01). The comet assay demonstrated that the exposed workers had greater DNA damage, reflected by a longer tail length and higher Olive tail moment, than control subjects (p<0.05). Conclusion: The current levels of PAHs emitted from the coke oven resulted in significant DNA damage. Both MN and comet assays can be employed as useful biomarkers in humans to evaluate and monitor the genotoxic effects associated with coke oven emission.

1861 ARE SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) ASSOCIATED WITH SYSTEMIC DISEASE CRITICAL IN EXPOSURE ASSESSMENT?

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Significant individual variation exists in systemic response to a xenobiotic exposure. The source of variation may be dependent upon single nucleotide polymorphisms (SNPs) in the highly conserved coding region of genes or in their regulatory control sequences (locus-control regions, promoter, intron, etc.) that may affect gene expression and function critical to xenobiotic metabolism. Currently, sources of individual genetic variation in response to exposure to xenobiotics are not accounted for in exposure and risk assessment models. Our goal is to investigate the contributions of individual genetic variations as well as other personal and environmental factors to systemic exposure to naphthalene (e.g., urinary metabolites, skin adducts) among 124 jet fuel workers. SNPs were genotyped using Affymetrix GeneChip Mapping 250K Sty array, and SNPs significantly associated with systemic exposure levels were identified using candidate gene and genome-wide association study in PLINK. Multivariate linear regression model was constructed in SAS to investigate the significance of SNPs and other workplace and individual factors. Our results showed that the incorporation of SNP variants increases overall R2, thus explaining more of the variability in systemic exposure levels than the non-genetic factors without an increase in the number of predictors in the exposure model. This research has allowed us to investigate the impact of individual metabolic differences due to gene expression in response to naphthalene exposure and to increase our knowledge on the potential role of individual genetic differences in exposure assessment.

1862 PROGRESS TOWARD ESTABLISHING QUANTITATION OF ONCOGENIC POINT MUTATIONS AS AN ENDPOINT FOR ASSESSING CARCINOGENIC POTENTIAL.

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Whether or not a chemical induces mutation, the level of mutation induced at different doses, and the mechanism(s) of mutation induction are critical issues for evaluating the cancer risk associated with chemical exposures. We are developing the use of specific oncogene and tumor suppressor gene mutations as cancer-relevant endpoints with which to address these issues. Studies examining mutation response to mutagen exposures have demonstrated specific oncogenic base substitution mutations can be quantified (as mutant fraction, ratio of mutant to wild-type allele) using a sensitive DNA-based method called allele-specific competitive blocker PCR (ACB-PCR). Using four different mutagens [4-aminobiphenyl, simulated solar light (SSL), benzo[a]pyrene (Ba[a]P), and azoxymethane] in model systems previously characterized in terms of rodent tumor response, we have shown that tumor-associated mutation induction can be detected at earlier times, and in one case “at lower dose”, than is needed to observe a significant tumor response. ACB-PCR has been used to describe the induction of p53 and K-Ras mutations as a function of dose of several known mutagens, including SSL, Ba[a]P, and aristolochic acid. Strategies based on the analysis of multiple mutations have been developed as a means to elucidate the mechanism(s) of oncogene or tumor suppressor gene mutation induction. By analyzing a mutation consistent with the mutational specificity of the chemical, along with a second “high level” spontaneous mutation (with different mutational specificity), the approach has the potential to distinguish “true” de novo induction from potential contamination or sampling mutation. Thus, ACB-PCR measurement of oncogenic point mutations is a promising and relevant endpoint for assessing the carcinogenic potency of chemicals.

1863 INTEGRATING HUMAN SOMATIC MUTATION BIOMARKERS INTO CANCER RISK ASSESSMENT.

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Somatic mutations in oncogenes, like K-RAS, are being developed as biomarkers of cancer risk for use in clinical settings, such as cancer screening, prognosis, and therapy selection. We are collecting data on levels of oncogene and tumor suppressor gene mutations (oncomutations) in normal human tissues and in neoplastic tissues, in order to define the levels of mutation that are indicative of disease state. Toward this end, allele-specific competitive blocker-PCR (ACB-PCR) has been used to measure mutant fractions (MFs) of particular oncomutations, such as those at K-RAS codon 12, in various human tissues and tumors. The MFs measured in normal tissues with high potential of developing onconation-derived tumors, such as colon and lung, were compared to the MFs in heart, an organ with low neoplastic potential. For K-RAS codon 12 GAT MF, for example, the level of mutation in normal lung and normal heart were found to be approximately equivalent (~1.5 x 10⁻³). Multiple oncomutations are being analyzed in a variety of different tissue and tumor types in order to identify which mutations will be the most useful mutational biomarkers of neoplasia for particular tissues. Analogous oncomutations are
being measured in experimental rodent models as endpoints for assessing the carcinogenic potency of chemical exposures. By integrating spontaneous levels of human oncomutations, and population variability in these levels, with spontaneous levels of cancer-relevant endpoint which may enhance our ability to predict human tumorigenic potency of chemical exposures. By integrating spontaneous levels of cancer-relevant endpoint which may enhance our ability to predict human tumor

1865 PHARMACOKINETICS OF 3, 5, 6-TRICHLORO-2-PYRIDINOL (TCPy), A CHLORPYRIFOS METABOLITE, IN RAT SALIVA.

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Novel non-invasive techniques are being developed for biological monitoring (biomonitoring) of a variety of potential toxicants, including pesticides like chlorpyrifos (CPF); and saliva has been suggested as an ideal body fluid to biomonitor. In order to be acceptable, there is a need to understand salivary pharmacokinetics of CPF metabolites for extrapolation of saliva measurements to whole body exposures. In this context, in vivo pharmacokinetics of 3,5,6-trichloro-2-pyridinol (TCPy), a major metabolite of CPF was quantitatively evaluated in rat saliva. Experimental results suggest that TCPy partitioning from plasma to saliva in rats is relatively constant over a range of varying physiological conditions. TCPy pharmacokinetics were very similar in blood and saliva (area under the curve (AUC) values were proportional and elimination rates ranged from 0.007 to 0.019 h⁻¹) and saliva/blood TCPy concentration ratios were not affected by TCPy concentration in blood (p = 0.35) or saliva flow rate (p = 0.26). The TCPy concentration in saliva was highly correlated to the amount of unbound TCPy in plasma (r = 0.96), and the amount TCPy protein-binding in plasma was substantial (98.5%). The median saliva/blood concentration ratio (0.045) was included as a saliva/blood TCPy partitioning co-efficient within an existing physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for CPF. The model accurately predicted TCPy concentrations in saliva over a range of blood concentrations. These studies suggest that saliva TCPy concentration can be utilized to ascertain CPF exposure, and it is envisioned that the PBPK/PD can likewise be used to estimate CPF dosimetry based on the quantitation of TCPy in spot saliva samples obtained from biomonitoring studies. Supported by Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (CDC/NIOSH) grants R01 OH088173 and R01 OH003629.

1866 ELABORATION OF MICROPLATE SPECTROSCOPIC METHODS FOR BIOMONITORING OF WARFARE ORGANOPHOSPHATES SOMAN AND RUSSIAN VX.


The scale of organophosphates (OPs) application demands strict control and safety of the personnel, population and environmental objects. The chemical methods are laborious, time consuming and require costly high precision equipment; also, they are relatively insensitive compared to biochemical ones. Two microplate spectroscopic methods for determination of organophosphates, based on inhibition of acetylcholinesterase (AChE) activity, have been elaborated and evaluated for determination of the chemical weapon agents soman and Russian VX (RVX). The limits of quantification were lower for the Ellman method, though the sensitivity coefficients were in favor of the Hestrin method. The effects of the main hydrolys products were consistent for the two methods. The main components of deconaminating solutions showed differential effects: while monoethanolamine had no influence upon results obtained by either method, hydrogen peroxide interfered with the Ellman method at far lower concentrations that with the Hestrin method. We have also adapted the method of Ellman to microplate version with human neuroblastoma cell line SH-SY5Y as a carrier of AChE, and tested it with soman and RVX, as well as their mixtures. The limits of quantification have been found to be (1±0.2)*10E-8 mg/ml for soman and (0.5±0.2)*10E-8 mg/ml for RVX. The linear ranges were determined to be 0.5*10E-8 to 8*10E-8 mg/ml for RVX and 2*10E-8 to 10E-7 mg/ml for soman. An additive character of action of the two OPs has been revealed. For biomonitoring purposes we are interested in the following advantages of the methodology developed: 1) High productivity and efficiency; 2) Elimination or reduction of false-positive and false-negative results; 3) Reduction of amount of samples for biochemical and possible chemical analyses; 4) Reduction of time necessary for analyses to be done.
used in the study contained 20 mg active) which is dispersed via a fan into the immediate personal area. In this study the air concentrations and surface deposition of the active were measured when the device was placed on a mannequin wearing a cotton longjohn (dosimeter) in an outdoor location. A second mannequin re- representing a child was placed next to the mannequin with the device. Air sampling tubes connected to air sampling pumps were placed at the breathing zone of the two mannequins. Air samples were collected at 0-3 and 3-6 hours. At the end of the 6 hours, 5cm x 5cm sections from the longjohns dosimeter were collected from 7 different locations. The samples were put in the refrigerator at 3-4°C until the 6 hours, the devices were wiped with gauze wetted with diethyl sulfoxide (DSS) or isopropyl alcohol (IPA) to determine potential for active transfer from handling the device. The results of the study clearly showed that active air, dosimeter, and device deposition concentrations were very low with most values below the limit of quantitation (LOQ). All samples were analyzed by HPLC/MS/MS with an LOQ of 0.08ng/L of air, 0.535μg/25cm² for dosimeters, and 1.07μg/sample for DSS wipes. IPA wipes of the device all had measured residues just above the LOQ. This exposure information was used in a product risk assessment demonstrating large safety margins from product use thus far exceeding minimum safety standards.

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When long-term consumption (sub-chronic and chronic exposures) are of interest rather than consumption on any given day (acute exposure), the long-run average daily intake of a food cannot be observed in short term cross-sectional surveys such as the 24-hour dietary recall in the National Health and Nutrition Examination Survey (NHANES). Using a probabilistic approach the frequency of intake of fish and shellfish over a 30-day period from the NHANES 1999-2006 were combined with the 24-hour dietary recall data from NHANES 2003-2004 to estimate the long-term average daily intake of fish and shellfish for the overall US population and sub-populations of children age 1 to 10y, males and females age 11y to 15y, 16y to 20y and 21y+. Over 300 NHANES food codes were mapped to 31 fish and shellfish categories and for each category a weighted distribution of amounts consumed per eating occasion was generated. The types of fish most frequently consumed by children age 3-5 years, in descending order, are tuna, breaded fish, and salmon. The types of shellfish most frequently consumed by children age 3-5 years, in descending order, are shrimp, crab, and clam. Among children, the highest usual daily intake of all fish and shellfish occurred in children age 6-10 years, with a per user mean intake at 8.9 g/day. When intake is normalized by bodyweight, children 2 years of age had the highest usual daily intake of all fish and shellfish, with a per user mean intake at 0.39 g/kg-bw/day. Males age 18 - 20 years showed the highest per user mean intake of fish and shellfish at 21.8 g/day. Fish and shellfish are excellent sources of nutrients, but exposure to environmental contaminants can also occur from fish consumption. Usual intake estimates on this approach can be used to better assess nutrient intakes as well as chronic exposure to environmental contaminants from fish and seafood sources.

1870 IDENTIFICATION AND QUANTIFICATION OF THE PROHAPTEN, DEHYDROABIEATIC ACID IN NON-LATEX SURGICAL AND EXAM GLOVES.
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Allergic contact dermatitis (ACD) is a well recognized health problem commonly associated with the use of latex and nitrile gloves. Vulcanization accelerators, including zinc dithiocarbamates and mercaptobenzothiazole, found in latex and nitrile gloves have been identified as major etiologic agents of glove-associated ACD. Recently, accelerator-free neoprene-type (polychloroprene, polychloroprene) surgical and exam gloves have been introduced to prevent both Type I and Type IV allergic reactions. During assessment of gloves used by an ACD patient, disproportionated resin was identified in dichloromethane extracts. Dehydroabietic acid (DHA), dihydroabietic acid and other pimaric/isopimaric species were observed by gas chromatographic mass spectrometry (GC-MS). DHA is a Type IV prohapten that can be air oxidized to the active allergenic form. Four different brands of neoprene-type gloves were purchased and DHA content quantified. A latex surgical glove was used as a negative control. All neoprene-type gloves contained DHA ranging from 7 to 31 μg/mgl glove. A preliminary leaching study of DHA from one glove brand suggests that small amounts of DHA can leach into artificial sweat (approx. 1 μg/mL). Oxidized DHA was not observed in any of the gloves assayed. It is concluded that DHA exposure may occur from neoprene-type glove use, although; potential association with glove ACD has not been established.

1871 EVALUATION OF THE IMPACT OF SOIL EXPOSURES TO DIOXIN-LIKE COMPOUNDS ON BODY BURDEN IN A POPULATION IN MIDLAND MICHIGAN.
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The University of Michigan undertook a study (UMDES) to measure dioxin-like compounds (DLCs) in serum of individuals in the Midland, Michigan area and to identify factors that explained variation in the DLC serum levels. UMDES included a questionnaire that focused on activities associated with exposure to DLCs, and collection of soil, household dust, and serum from study participants for DLC analysis. Study participant DLC serum levels were generally similar to the control population even though soil DLC levels were higher in the Midland area than in the control area. Linear regression models developed to identify predictors of serum DLC concentrations indicated that demographic factors (age, sex, race, BMI, etc) explained most of the variation, while soil and house dust DLC concentrations were found not to explain any of the variation. Because some have expressed concern that UMDES may have failed to characterize highly exposed individuals, we undertook an analysis to address this. We developed high-end and central tendency exposure scenarios based on the characteristics of the Midland population, including a worst-case lifetime scenario that explicitly addressed higher intake of DLCs during the childhood years. Intakes of DLCs from exposure to soils were estimated for each scenario using conservative U.S. EPA exposure assumptions and body burden was predicted for soil intakes as well as for background exposures using the Carrier/Aylward model. Modeled serum DLC concentrations were compared to the serum DLC measurements from the UMDES control population and NHANES. Predicted for the upper bound soil concentration evaluated, the predicted serum TEQ associated with exposure to soil was found to be small (0.53 to 2.38 ppt lipid across the four exposure scenarios evaluated). Modeled total serum TEQ values were also well within the range of background levels observed in UMDES control and NHANES populations. These results further support the UMDES findings that soil exposures contribute very little to serum TEQ.

1872 ASSESSMENT OF THE IMPACT OF VARIOUS SOIL CLEANUP LEVELS ON SERUM CONCENTRATIONS OF DIOXIN-LIKE COMPOUNDS IN HUMANS.
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One of the components of the EPA’s Science Plan for Activities Related to Dioxins in the Environment is an evaluation of information concerning the basis for soil cleanup levels for dioxin-like compounds (DLCs), culminating in the recommendation of updated interim DLC soil cleanup level. We believe that a critical aspect of establishing an updated soil cleanup level is an assessment of the impact that exposures to DLCs in soils have on human body burden. To accomplish this, we evaluated the impact of soil exposure on DLC body burden for numerous exposure scenarios, including a worst-case lifetime scenario that explicitly addressed higher intake of DLCs during the childhood years. EPA exposure assumptions were used in conjunction with a range of soil concentrations (9-2000 ppt) to calculate lifetime average daily doses for each exposure scenario. The Carrier/Aylward model was then used to predict body burden for each scenario and soil concentration combination. Modeled serum DLC concentrations were compared to the serum DLC measurements in the general US population as reported in NHANES and were found to be within their respective age-specific ranges in NHANES for all scenarios, even at the highest soil concentration evaluated. Modeled serum DLC concentrations were also compared to those predicted based on acceptable intakes established by regulatory agencies. Even at 2000 ppt in soil, modeled serum concentrations were below those based on intakes associated with ingestion of the lower bound of the acceptable range of TDIs established by WHO (1 pg TEQ/g lipid). Soil concentrations had to be in excess of 1000 ppt before serum DLC concentrations fell outside of those associated with the EPA acceptable risk range of 10-4 to 10-6 (based on a cancer slope factor of 156,000). These findings provide important information for understanding the impact of soil clean up levels on human body burdens.

1873 RE-INTERPRETING HISTORICAL EXPOSURE DATA ASSOCIATED WITH THE USE OF CHRYSOTILE-CONTAINING JOINT COMPOUND.
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Chrysotile-containing joint compound (JC), i.e., fibers in a calcium carbonate- or calcium sulfate-based matrix, was commonly used in residential and commercial construction through the mid 1970s; however, these products have not been man-
ufactured in the US for more than 30 years. Little is known about actual human exposures to fibers that may have resulted from the use of chrysotile-containing JC, because few exposure and no health-effects studies have been conducted specifically with these products. Data from two of the largest exposure studies suggest that short-term airborne fiber concentrations during sanding generally ranged from 5 to 10 f/cc. However, historic studies provided only limited information regarding the products tested, methods used, and exposure conditions, other than samples were collected as TSP on IH cassettes and prepared and analyzed by methods that pre-ceded the current NIOSH method. Therefore, small-scale studies were undertaken to re-create and test a chrysotile-containing calcium carbonate-based JC from an original 1960s formulation, along with a current asbestos-free JC. These studies characterized the respirable and TSP fractions of chrysotile-containing JC as the number of chrysotile fibers/mass of dust generated during sanding and characterized both materials in terms of amount of respirable dust emitted during this activity. These data were combined with previously published field data on respirable dust concentrations during sanding to estimate the airborne chrysotile concentration associated with sanding chrysotile-containing JC. Study results indicate that historic measurements overestimated potential exposures to fibers in respirable dust by a factor of 4 or more, primarily due to sampling and preparation artifacts unique to this material: 1) some portion of the chrysotile fibers were associated with the non-respirable fraction being collected on the IH cassette (3x), and 2) preparation using the historical method released previously unavailable fibers from matrices (1.5x).

Comparison of Paired Exposure Measurements of Libby Amphibole Asbestos in Air Measured by Direct and Indirect Preparation Analyses.


Measuring asbestos in air collected at Libby, MT is necessary to estimate exposures of residents to Libby Amphibole asbestos (LA) and to evaluate associated risks. The choice of analytical preparation steps employed in the measurement of airborne asbestos may impact the outcome of results. In particular, direct analysis of air filters without further preparation has conventionally been the preferred method for estimating asbestos concentrations in air. However, air sampling in residential settings is particularly challenging in that additional particles can interfere with the asbestos measurements and may necessitate analysis via indirect preparation. Therefore, we performed a pilot study to: 1) determine whether exposure measurements differ significantly for LA when measured by direct and indirect analyses; and 2) evaluate the need for immediate disqualification of any data generated through indirect analysis. The pilot study evaluated 31 air samples, known to have measurable levels of LA, and analyzed paired samples using transmission electron microscopy (TEM) by both direct analysis and indirect preparation analysis methods. When compared pair-wise, indirect preparation samples were: a) statistically higher (p<0.05) than the matched direct preparation samples in 14 of 31 (45%) cases; b) statistically lower in 7 of 31 (23%) cases; and c) not statistically different in 10 of 31 (32%) of the cases. The Wilcoxon signed rank test indicates that the paired data sets (direct vs. direct) are not significantly different from each other. The result of this work confirmed the adequacy of our approach at the Libby Asbestos Superfund Site: TEM measurements of LA in air are reliably analyzed using the TEM analytical method, when feasible, but obtaining estimates of LA exposures using the indirect preparation analysis method also may be reasonable given some of the challenges of sampling in environmental settings.

Prevalence Rates of Pleural Abnormalities Among Populations With Environmental Asbestos and Non-Asbestos Exposures.

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The rate of pleural abnormalities such as pleural plaques, thickening, calcification, pleural fibrosis and pleural effusion from occupational asbestos exposures has been heavily studied. Even though occupational exposures are clearly implicated, the background prevalence rates of pleural abnormalities and those related to environmental asbestos exposures are not well-established and vary considerably between populations. In order to determine the reasons for such widely-varying prevalence rates and characterize typical rates among different population exposures, we conducted a literature review of studies reporting prevalence rates of pleural abnormalities resulting either from environmental asbestos exposures or non-asbestos exposures. The categories of exposure in the studies identified ranged from populations exposed to erionite, people living in houses that had asbestos in the stucco or the whitewash on the walls, populations living near a mine or factory handling asbestos, populations living in areas of naturally occurring asbestos, populations not exposed to asbestos or erionite (background), and the general population. There were also studies that reported prevalence rates of pleural abnormalities based on autopsies conducted in accident victims in whom occupational exposures were ruled out by the investigators. Overall, the rates within and between these exposure categories varied substantially due to the varying definition of “pleural abnormality” and the study’s ability to clearly define the exposure, or lack of exposure, in the population studied.

An Evaluation of Health Risks Associated with Exposure to Erionite Fibers in North Dakota.

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Exposure to erionite, a zeolite group mineral, is associated with malignant mesothelioma in both human epidemiological and experimental animal studies. The toxicity of erionite was first recognized in the 1970’s when an outbreak of malignant mesothelioma was confirmed in three rural villages in Cappadocia, Turkey. In 2006, the North Dakota Department of Health and Geological Survey initiated an investigation of naturally occurring erionite deposits used for gravel sources for rural county roads for several decades. The U.S. EPA was asked to investigate and evaluate the possibility that erionite in the gravel may pose a threat to residents. Both stationary and activity-based air samples were collected to determine (1) if the fibers were similar to those found in Turkey and (2) the concentration that could be inhaled. They were analyzed by transmission electron microscopy and binned according to length and aspect ratios. The erionite fibers from North Dakota were found to have a similar morphology and chemistry to those from Turkey. Exposure and risk to the fibers was estimated using the U.S. EPA’s Framework for Investigating Asbestos-Contaminated Sites. Cancer and non-cancer toxicity values for asbestos were used since no quantitative toxicity values exist for erionite. The modeled risk results suggested that people who routinely drive on the gravel roads exceed EPA’s decision criteria for both cancer and non-cancer risk. A medical screening study was conducted to determine the prevalence of chest radiographic abnormalities, including pleural and interstitial changes, among residents of western North Dakota thought to have the highest exposures to gravel roads containing erionite. The fiber structure data, modeled risk results, and medical study findings were used in a weight of evidence approach to make recommendations for actions to reduce exposures to erionite fibers.

Characterization of Model Uncertainty in Asbestos Cancer Risk Calculations.

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The U.S. EPA Office of Solid Waste and Emergency Response (OSWER) has developed a Microsoft Excel® spreadsheet tool for calculating the risk of cancer following inhalation exposure to asbestos in accord with current U.S. EPA guidance (U.S. EPA 2008). This spreadsheet also implements risk calculations for several alternative asbestos cancer risk models. This includes a version of the current risk model updated with new mortality statistics, as well as the risk models developed by Hodgson and Darnton (2000) and Berman and Crump (2008). The purpose of these alternative calculations is to provide risk assessors and risk managers with a way to characterize “model uncertainty” (i.e., the variation in risk estimates as a function of which risk model is used). The user specifies the concentration of the asbestos atmosphere in units that are appropriate for each of the risk models, and provides the exposure parameters for the site-specific exposure scenario of interest. The spreadsheet returns the risk estimates for EPA’s current model, as well as the other risk models. This spreadsheet tool will be used to compare model-specific risk estimates for several alternative case studies in order to illustrate how the tool can be used to better characterize model uncertainty in asbestos risk assessments.

Urinary Biomarker Panel Selection Indicative of Early Subclinical Renal Injury to Toxin Exposures.

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A urinary protein expression profile was conducted to 10 markers in a time/dose response study using the nephrotoxin D-serine. Male CDF®(F344)/CrBHR rats were dosed intraperitoneally with 0, 200 or 500 mg/kg D-serine in 0.9% saline. Urine
was collected prior to dosing and at 12 hour intervals for a total of 168 hours. Urine samples were assayed by ELISA for clusterin, retinol binding protein (RBP) 4, heme oxygenase (HO)-1, osteopontin (OPN), Yb1 (μ) glutathione S-transferase (GST), α glutathione S-transferase (α GST), metalloproteinase tissue inhibitor (TIMP)-1 and β2-microglobulin. Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (Kim-1) were assayed by Meso Scale Discovery (MSD) Multi-Spot® Assay. Biomarker levels remained constant for control animals throughout the time course. However, for animals dosed with 200 and 500 mg/kg D-serine, significant increases were observed with peaks at 12 hours post-dose (HO-1, Yb1 GST and α GST), 24 hours post-dose (clusterin, RBP4, TIMP-1 and β2-microglobulin), 96 hours post-dose (Kim-1 and NGAL) or 120 hours post-dose (OPN). Biomarkers returned to baseline levels at 36 hours (Yb1 TIMP-1 and β2-microglobulin) for animals dosed with 200 and 500 mg/kg D-serine, and significantly lower expression upon nephrotoxin exposure. Expression profiles indicate that this protein set differed in maximal response times. Their collective detection in urine is a potential noninvasive strategy to determine early onset of low level subclinical kidney damage in response to toxin exposures, ultimately leading to development of rapid field monitoring for the prediction of health hazards associated with chemical exposure.

**1879 TRANSPLACENTAL DISTRIBUTION OF METALS AND THEIR INTERACTIONS ASSESSED BY BIOMONITORING IN MOTHER/CHILD PAIRS.**

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Exposure of the fetus to (heavy) metals has been associated with adverse health outcomes including developmental toxicity. However, few data exist on the transplacental passage of metals and their interaction with each other in the maternal-fetal unit. In our study, venous and umbilical cord blood samples from 90 mother/child pairs were studied for exposure to multiple heavy metals, essential minerals and trace elements. Smoking status was assessed by cotinine in urine. Lead (Pb) showed the highest median concentration of heavy metals in maternal samples (11.5 μg/L) followed by nearly equal concentrations of mercury (Hg, 0.44 μg/L) and cadmium (Cd, 0.34 μg/L). Smokers showed higher Cd levels than non-smokes (0.73 vs. 0.29 μg/L, P<0.001). Slightly but significantly lower levels of Pb were observed in fetal blood (10.3 μg/L, P<0.004), whereas Cd was strongly reduced (0.05 μg/L). In contrast, higher concentrations of Hg were detected in fetal samples (1.48 μg/L, P<0.0001). No associations between maternal and fetal concentrations for Pb, Mn and Hg were found for Cd, Cu, Fe and Zn. Exposure to heavy metals (single or in combination) did not influence the levels of essential minerals such as Zn. In conclusion, the placenta provides a barrier for Cd, Cu and Zn, whereas Fe, Pb and Se enter the fetal environment unaffected. Mn and Hg are unequivocally transported to the fetus resulting in increased exposures compared to the mother. However, homoeostasis of essential elements remains unaffected by exposure to heavy metals at low exposures. Overall, our results contribute to the risk assessment of heavy metals and adverse health outcome in the most vulnerable population, the fetus.

**1880 SURVEILLANCE FOR SYSTEMIC EFFECTS OF METALS AND OTHER MATERIALS RELEASED FROM RETAINED EMBEDDED FRAGMENTS IN U.S. SOLDIERS.**

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Concern has heightened regarding long term health effects associated with embedded fragments in soldiers. In the past, fragments embedded in muscle tissue were thought to be relatively inert, however recent work has shown that veterans with embedded depleted uranium (DU) fragments have elevated blood and urine uranium levels more than 18 years after injury involving DU munitions during the first Gulf War. This finding is supported by studies showing release of metals from certain types of medical implants. To better understand and prevent health problems resulting from retained metal and non-metal fragments in soldiers, the Department of Veterans Affairs has established a program charged with developing clinical management guidelines for embedded fragments. These will be based on results from analysis of fragment content, health surveillance and biomonitoring of veterans with prolonged systemic exposure to chemicals released from fragment material over time. Chemical characterization of over 400 removed fragments has shown that most are metal alloys (83%) while others are different types of organic material, plastics, wood and stones. Based on this information and knowledge of the toxicity of metals, a biomonitoring protocol utilizing primarily urine has been developed to characterize systemic exposure to the following carcinogenic and cytotoxic metals: Al, As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, U, W and Zn. Customized health surveillance and management guidelines will be developed for veterans with chronically elevated excretion of specific metals using biomarkers of potential effects of the metal(s) of concern. Biomonitoring protocols for compounds released from non-metallic fragment materials, such as isocyanate, phosphates and acrylics, will continue to be developed as our knowledge of the breakdown of fragments embedded in muscle tissue increases. Supported by Department of Veterans Affairs and the Armed Forces Institute of Pathology.

**1881 DOD IMPACT ASSESSMENT AND MANAGEMENT OF NAPHTHALENE-RELATED RISKS.**


The Department of Defense Chemical and Materials Risk Management Directorate is using a scan-watch-action process to identify, rank and manage risks associated with emerging contaminants. Naphthalene is characterized as a likely human carcinogen by the NTP and in the EPA's most recent draft health risk assessment. Thus, naphthalene-related environmental health regulations are evolving. The potential impacts have been assessed, using multi-criteria decision analysis, for five of the Department's functional areas. One of the areas of concern is exposure to naphthalene among fuel handlers. To determine whether these exposures present unacceptable risk, the Army Research Office awarded a Small Business Innovative Research Project for the development of a miniature real-time naphthalene sensor. NIOSH's Biomonitoring Team and Investigators from the Army Research Institute for Environmental Medicine, UC-Davis and the Army Corps of Engineers are collaborating on a second DOD-funded project. This project will validate the prototype sensor as a dosimeter by defining correlations between measured exposures and biomarkers of exposures to be-collected from military fuel handlers. To date, naphthalene specificity with sensitivity of 0.5 mg/m3 has been demonstrated and definition of the firmware chemometrics is underway. Implementation of the human subjects research protocol is pending institutional review boards' approval.
Bone Pb measured by a K x-ray fluorescence (KXRF) technology has been used as a biomarker for epidemiology and toxicology study on Pb for over two decades. However, there are only a few labs worldwide that possess the KXRF technology, principally because of its infrastructural demands. This study is conducted to investigate the feasibility and methodology of applying a portable XRF technology in vivo measurement of Pb in bone. The advantages of this XRF system over the KXRF system are that it is portable, fast, and user-friendly. We set up and optimized such a system for bone Pb measurement. A 7.5 MHz diagnostic ultrasound scanner was used to measure soft tissue thickness to account for X-ray attenuation. We developed an algorithm to calculate the Pb concentrations, calculated the minimum radiation dose delivered to the subject by Monte Carlo simulations, and compared the results of measurements of Pb concentrations in tibia bone in humans in vivo using the standard KXRF technology and the portable XRF technology with a Spearman correlation of 0.79 (P = 0.01). This suggests that the bone Pb concentration obtained from portable XRF would be useful for epidemiological studies of bone lead concentration and health outcomes. The total body effective dose delivered to the human subject is about 1.5 μSv. In conclusion, portable XRF technology can be used for in vivo bone Pb measurement with sensitivity comparable to the previous KXRF technology, and with tremendous advantages over KXRF of speed and portability.
Diethylphosphate (DEP), diethylthiophosphate (DETP) and 3,5,6-trichloro-2-pyridinol (TCPy) are products of both in vivo metabolism and environmental degradation of the insecticide chlorpyrifos (CPF) and are routine urinary biomarkers of exposure. However, biomonitoring of TCPy, DEP and DETP may be reflective of an individual’s contact with both the parent pesticide and exposure to their metabolites in the environment. In the current study, simultaneous dosing of 13C- or 2H-isotopically labeled CPF (13C-labeled CPF, 5 13C on the TCPy ring; or 2H-labeled CPF, diethyl-d10 (deuterium labeled) on the side chain) were exploited to compare the pharmacokinetics of CPF with TCPy, and DETP. The study objective was to quantitatively evaluate the pharmacokinetics of the individual metabolites relative to their formation following a dose of CPF. Individual metabolites were co-administered (gavage) with the parent compound at equal molar doses (14 μmol/kg -5mg/kg CPF). Major differences in the pharmacokinetics between CPF and metabolite doses were observed within the first 3 h of exposure, due to the required metabolism of CPF to include TCPy and DETP. Nevertheless, once a substantial amount of CPF has been metabolized (≥ 8 h post-dosing) pharmacokinetics for both treatment groups and metabolites were very comparable. Urinary excretion rates for orally administered TCPy and DETP relative to 13C-CPF or 2H-CPF derived 13C-TCPy and 2H-DETP were consistent with blood pharmacokinetics, and the urinary clearance of metabolite dosed groups were comparable with the results for the 13C- and 2H-TCPy groups. Since the pharmacokinetics of the individual metabolites were not modified by co-exposure to CPF; it suggests that environmental exposure to low dose mixtures of pesticides and metabolites will not impact their pharmacokinetics. (Supported by CDC/NIOSH grants R01 OH008173 and R01 OH003629).

DDT (from its trivial name, dichlorodiphenyltrichloroethane) is one of the most well-known synthetic pesticides. It has potent insecticidal properties, where it kills by opening chloride ion channels in the neurons, causing them to fire spontaneously leading to spasms and eventual death. o,p'-DDT is a DDT isomer that can induce cholinergic responses that cause a drop in blood pressure, and lead to spasms and eventual death. However, the effect of o,p'-DDT on aromatase is unclear. Therefore, we investigated the effect of o,p'-DDT on aromatase expression in human breast cancer cells. We also studied whether cyclooxygenase (COX) 2 was involved in o,p'-DDT-mediated aromatase expression. o,p'-DDT induced aromatase protein expression in human breast cancer MCF-7 cells. In addition, o,p'-DDT enhanced aromatase gene expression and activity of enzyme and promoter in MCF-7 cells, o,p'-DDT also markedly increased the levels of COX-2 protein levels in MCF-7 cells. o,p'-DDT induced the production of prostaglandin E2 (PGE2) and the gene expression of PGE2 (EP2 and EP4) receptor. Moreover, o,p'-DDT induced cyclic AMP response element (CRE) activation, cAMP level and (PGE2) and the gene expression of PGE2 (EP2 and EP4) receptor. Moreover, o,p'-DDT induced aromatase and o,p'-DDT-induced aromatase was correlated with COX-2 up-regulation mediated via PKA and PI3-kinase/Akt signaling pathways in breast cancer cells.

Endosulfan is an organochlorine compound that is used as an insecticide and acaricide. This colourless solid has emerged as a highly controversial agrichemical due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor. However, its mechanism on inflammation of macrophages is unclear. This study examined the effects of endosulfan on cyclooxygenase (COX-2) expression and examined the molecular mechanism in macrophages. Exposing macrophages to endosulfan induced the production of prostaglandin E2 (PGE2). In addition, endosulfan enhanced COX-2 gene expression, protein level and luciferase activity. The transient transfection and electrophoretic mobility shift assays with the NF-κB-binding sites showed that the NF-κB transcription factor mediated the endosulfan-induced increase in the expression levels of COX-2. These results show that endosulfan stimulates the production of PGE2 and COX-2 expression and up-regulate the gene expression levels through NF-κB transactivation. Overall, these results suggest that endosulfan has inflammatory potential.

Ethylenebisdithiocarbamate (EBDC) pesticides maneb, mancozeb and zineb are widely used in the prevention of fungus on a variety of plants and crops. While these agents are reported to possess low human toxicity, recent scientific studies have suggested that human toxicity to these agents do occur. These agents are rapidly degraded and their main metabolite is ethyleneurea (ETU), a known carcinogen, teratogen and goitrogen. As metal containing compounds, participation in Fenton-like reactions might also contribute to oxidative stress. In addition, binding of metal moieties to critical sulphhydryl groups in enzymes is a common mechanism of metal induced toxicity. The purpose of this study was to investigate the acute toxicity of these three EBDC pesticides in human Caco2 and Ht-29 colon cells. Each cell type was grown to subconfluency and treated with concentrations of each agent ranging from 0.0 to 0.39 μM for a period of 24 hrs. Cell viability was assessed by MTT assay. HT-29 cells showed significant decreases in viability in concentrations of 400 and 200μM for both maneb and mancozeb treatments and Caco2 cells in concentrations ranging from 400-100μM. For both compounds. In sharp contrast, zineb treatment showed no significant decrease in cell viability in either cell type in treatment up to 3.2 mM. Both phase contrast and scanning electron microscopy were performed to confirm viability results with maneb and mancozeb treatment and to observe morphologic changes observed with exposure. Microscopic studies confirm significant loss of viability observed with MTT analysis. Cell damage such as blebbing, decreased cell attachment fibers and disruption of cell structure, was observed in these groups. We conclude that maneb and mancozeb treatment results in toxicity in cells tested and that zineb possesses lower toxicity in these cell types. These data suggest that the metal moiety of EBDC pesticides may play a key role in toxicity.

The aim of study was to determine the contamination in the milk of cattle and goat reared in pesticide spraying areas of Faisalabad, Pakistan. Because no such published information is available in this region. The milk was collected from villages situated within the radius of 25-35 Km on four different localities (Jhang, Animpur, Sittana, and Shiklepura roads) in the Northeast and Northwest of city during winter and spring seasons of 2007-08. Five pesticides (cyhalothrin, endosulfan, flubopyrin, cypermethrin and methyl parathion) were analyzed in the collected cattle milk (n=240) and goat milk (n=240) with solid phase microextraction and high performance liquid chromatography techniques. The residue analysis revealed that 46% and 50% samples were contaminated with pesticides in cattle and goat milk, respectively. The concentration of cyhalothrin and cypermethrin was higher in the milk collected from northeast (0.401 ± 0.01 mg/kg and 0.047 ± 0.01 mg/kg respectively) as compared to the northwest of city (0.310 ± 0.03 mg/kg and 0.025 ± 0.007 mg/kg respectively). The milk samples collected during the winter season were found to be relatively more contaminated with pesticides as compared to spring. All the pesticides except endosulfan exceeded the maximum residual limits (MRL) established by the international health regulatory agencies, while methyl parathion was not detected in any milk sample. The species comparison revealed that only endosulfan residues were significantly (P<0.05) different in milk of cow and goat. About 19-25 % milk samples surpassed the MRL for cypermethrin, cyhalothrin and chlorpyrifos in both species. These findings suggest the need to create awareness in dairy farmers regarding the avoidance of pesticide residues in milk. (This work was supported by the Higher Education Commission, Islamabad, Pakistan.)
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The CYP1A subfamily of Pf50s may play an important role in the metabolism of critical environmental chemicals including chemical carcinogens. The genetically determined susceptibility to chemically induced cancers may depend on the metabolic balance of phase I and phase II enzymes. Relatively little is known about the effect of pyrethroids on drug metabolizing enzymes. From this point of view and because of metabolism may be an important determinant of pyrethroid toxicity, this study was designed to establish the potential joint effect of the insecticide pyrethroid lambda-cyhalothrin on CYP1A1, CYP1A2 and glutathione S-transferase enzymes in rat liver. Male Wistar rats were treated with lambda-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 then centrifuged. Aliquots of cytosolic fraction for glutathione S-transferase enzyme determinations and microsomal pellets for determinations of O-demethylation of methoxyresorufin (MROD) and O-deethylation of ethoxyresorufin (EROD) were causing immunotoxicity. In this study, we investigated the effects of two organophosphates and their metabolites: Chlorpyrifos, Chlorpyrifos-Oxon, 3,5,6-Trichloro-2-pyridinol, Isofenphos and Isofenphos-des-N-isopropyl on the cellular viability. Results demonstrated that lambda-cyhalothrin induced some members of CYP1A. For pyrethroid-treated animals, the percentage changes compared to control were: EROD (CYP1A1): 295%, P < 0.001; MROD (CYP1A2): 159%, P < 0.001. Moreover, lambda-cyhalothrin reduced glutathione S-transferase enzyme activity; for pyrethroid-treated animals, the percentage change compared to control was 23%, P < 0.01. These findings may provide a valuable contribution for risk assessment of this insecticide. Further studies on the regulation of the CYP1A subfamily by pyrethroids would be desirable. Work supported by projects No. CCG07-UCM/AGR-2618, No. UCM-BSEH GR58/08 & Consolider-Ingenio 2010 FUN-C-Food No.CSD2007-063 (MIP, Spain).

Organophosphates are ubiquitous chemicals used to control pests in urban and rural settings as well as on crops. Several published works have demonstrated that our foods and our bodies contain residual levels of these compounds. Moreover recent studies indicate that organophosphate pesticides may exert a myriad of effect ranging from acting as genotoxic agents, developmental neurotoxicants to possibly acting as neurobehavioral toxicants. Exposure estimates and PBPK modeling of chlorpyrifos (CPF) in rats and humans. Ties No. CCG07-UCM/AGR-2618, No. UCM-BSCH GR58/08 & Consolider-Ingenio 2010 FUN-C-Food No.CSD2007-063 (MIP, Spain).

Pesticide use and the potential public health hazards associated with exposure have resulted in continuing debate. The objective of this study is to evaluate urinary biomarkers identified in the U.S. population that are indicative of exposure to select pesticides. These biomarkers are a metabolite of the parent pesticide. This pilot study was conducted using the 2001-2002 National Health and Nutrition Examination Survey (NHANES) data to determine urinary biomarkers for chlorpyrifos, chlorfenoprofen, and pyrethroid exposure in a subset of the U.S. population (N=3057). Metabolites associated with acetochlor, carbulfuran, DEET, Diazinon and Metachlor were detected in less than 50% of the study samples, indicating an absence of biomarkers for these pesticides. Three pesticide specific biomarkers were detectable in more than 50% of the population: 58% of those sampled were positive for 2,4,6-trichlorophenol, a chlorophenol with a geometric mean urinary level of 2.61 μg/L (CI: 2.51-2.71); 79% were positive for 3,5,6-trichlorpyridinol, a metabolite of Chlorpyrifos with a geometric mean urinary level of 2.07 μg/L (CI: 1.98-2.17); and 77% were positive for 3-phenoxbenzoic acid, a metabolite of permethrin, with a geometric mean urinary level of 0.33 μg/L (CI: 0.31-0.35). Significantly higher levels of urinary 2,4,6-trichlorophenol and 3,5,6-trichlorpyridinol were found in Men (2.94 μg/L, CI: 2.77-3.11; 2.44 μg/L, CI: 2.29-2.61, respectively) than in Women (2.36 μg/L, CI: 2.23-2.48; 1.78 μg/L, CI: 1.67-1.90, respectively). Despite these differences, current epidemiological literature does not indicate that there is a public health hazard associated with the presence of these biomarkers in humans.

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Some noncholinergic toxicity of organophosphorus insecticides may result from their interference with protein synthesis. In this study, we investigated in vivo/ofo effects of an organophosphate diazinon [O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] on expression of proteins of the soluble fraction of liver and in yolk sac membranes of chicken embryos. The soluble fraction of livers in mice and in yolk sac membranes caused in vivo effects of the rats treated with a similar dose of AChE inhibition on rat and mice liver and yolk sac membranes such as an altered formation of several enzymes of glucose metabolism. Other diazinon-induced changes were species-specific, for instance a decrease in 3-hydroxoyanthranilate 3, 4-dioxygenase in mice and a reduced expression of prophyl 4-hydroxylase in chicken yolk sac membranes. The three above cited examples of diazinon impact on protein expression can be linked to specific toxic consequences: the altered expression of several enzymes of glucose metabolism to hyperglycemia in mice and humans, a reduced formation of 3-hydroxyanthranilate 3, 4-dioxygenase to altered NAD+ metabolism in mice, and a reduced formation of prophyl 4-hydroxylase to teratogenesis characterized by the muscle-skeletal

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Exposure estimates and PBPK modeling of chlorpyrifos (CPF) in rats and humans were conducted to assure that mechanistic neurobehavioral studies in rats use CPF doses that are relevant to those observed in Egyptian agricultural workers previously shown to have extensive neurobehavioral deficits. Exposure was monitored in Egyptian cotton workers (n=37) during 9 to 17 consecutive days of CPF application by measuring urinary trichloro-2-pyridinol (TCPy) levels and blood acetylcholinesterase (AChE) activity. Urinary TCPy levels ranged from 5.3 – 30.107 μg/g creatinine, while AChE levels were inhibited from 0 to 55%. With the assumption that the daily TCPy excreted in urine represents 70% of the daily absorbed dose of CPF, estimated average daily CPF doses for individuals were from 1.22 to 803 μg/kg/day, with single day exposures as high as 1.900 μg/kg/day. The average dose in applicators of 394 μg/kg/day resulted in an average depression in AChE of 44%. In comparative studies, Long Evans rats (n=9) were exposed to 0, 3 or 10 mg CPF/kg/day, s.c. for 7 days. CPF exposure produced a 31% and 53% decrease in blood AChE and a 9.7% and 64% decrease in brain AChE in the 3 and 10 mg/kg/day groups, respectively. PBPK modeling is generally consistent with the observed effects of exposure on urinary TCPy levels and AChE activity. Estimates of the human daily absorbed dose of CPF were within a factor of 2-3 of that in rat studies, which is consistent with the similar magnitude of AChE inhibition in rats and humans. Together, the results suggest that the rat model for CPF exposure is appropriate for future behavioral and mechanistic studies aimed at developing suitable biomarkers that are predictive of CPF induced deficits in humans. (This work was supported by NIEHS, grant #R01 ES016308 and U.S. EPA STAR grant R833454).

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Defects in chicken embryos. Identification of other proteins whose syntheses were altered by diazinon opens new venues to further investigations of the nontarget toxicity of organophosphorus insecticides. Acknowledgement: The protein analyses were carried out by the National Proteomics Center.

1897 HYDROLYSIS OF PARAOXON, DIAZOXON, CHLORPYRIFOS-OXON, AND DIHYDROCOUMARIN BY HUMAN SERUM PARAOXONASE 1 (PON1) AND RELATIONSHIP TO Atherosclerosis AND DIABETES.

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In addition to its ability to hydrolyze organophosphates, paraoxonase 1 (PON1) is reported to have antioxidant and cardiovascular system-protective properties. PON1, associated with the high density lipoprotein (HDL) particle, protects against atherosclerosis by hydrolyzing oxidized low density lipoproteins (LDL). The Q192R polymorphism influences PON1’s ability to hydrolyze these substrates. An analysis of enzyme activities of 200 human serum samples, from 120 Caucasians and 80 African Americans of both sexes (100 of each sex), was conducted along with comparisons to demographic and clinical information. These subjects were recruited from a cardiology clinic in north Mississippi and participants were characterized for a variety of demographic and clinical parameters, including the presence of atherosclerosis and diabetes. Functional genotype for the Q192R polymorphism was determined from plots of diazoxon hydrolysis vs. paraoxon hydrolysis. Caucasians had a higher level of diazoxon hydrolysis activity than African Americans while the reverse was true for paraoxon hydrolysis activity. A greater proportion of Caucasians displayed the QQ functional genotype than did African Americans, while the reverse was true for the RR functional genotype. The pattern of chlorpyrifos-oxon hydrolysis was more similar to that of paraoxon than of diazoxon. Caucasians had higher activities of dihydromucarin hydrolysis than African Americans. Statistically significant relationships (P < 0.05) were found between the presence of atherosclerosis and sex, age, statin use, smoking, body mass index, total cholesterol, LDL level, HDL level and diazoxon hydrolysis. Statistically significant relationships were found between the presence of type 2 diabetes and hypertension, total cholesterol and HDL level. The various measures are being investigated as possible biomarkers of disease risk. (Supported by NIH R21 E0815790)

1898 MECHANISM OF PARAQUAT-INDUCED PULMONARY TOXICITY IN TIME COURSE AND INTERVENTION OF PYRROLIDINE DITHIOCARBAMATE.

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The mechanism of paraquat (PQ)-induced pulmonary toxicity and potential therapeutic effect of pyrrolidine dithiocarbamate (PDTC) were studied. Male SD rats were divided into control group (6 rats, 0.9% NaCl gavage), PQ (80mg/kg, gavage) group and PQ+PDTC (100mg/kg, ip) group. On 1st, 3rd, 7th, 14th, 28th and 56th day after treatment, IL-1β, TNF-α, TGF-β1 and PDGF in serum were detected. Hydroxyproline (HyP) level and activity of NF-κB and expression of the connective tissue growth factor (CTGF) and α-smooth muscle actin (α-SMA) in lung tissues were measured. The lung pathological changes were observed and relationships with above indicators were evaluated. The level of TGF-β1 and TNF-α in PQ group significantly increased. The level of PDGF significantly increased on 7th, 14th, 28th and 56th day. The level of IL-1β significantly increased on 1st, 3rd, 7th day in PQ group. There were a significant decrease of IL-1β, TGF-β1, TNF-α and PDGF in PQ+PDTC group. The activity of NF-κB in lung tissue of PQ group significantly increased on 1st, 3rd, 7th and 14th day. There was a significant decrease in NF-κB activity on 1st, 3rd, 7th day in PQ+PDTC group. The content of HyP in PQ group was significantly higher on 14th, 28th and 56th day and its content showed lower in PQ+PDTC group. The expression of CTGF, α-SMA in PQ group increased gradually. The increasing extent of CTGF and α-SMA were gentler on the 3rd, 7th day, while their increasing was rapidly from the 14th to 56th day. Cytokine and NF-κB, CTGF and α-SMA could play an important role in paraquat-induced lung injury. PDTC may inhibit the expression of NF-κB and further reduce the production of cytokines and alleviate lung injury, which might have therapeutic effect in the clinic.

1899 LINKING DDT EXPOSURE TO DIABETES.

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There is growing evidence that exposure to DDT may be linked to diabetes. In order to gain insight into these possible relationships, a data mining approach was used to identify regulation pathways connecting the environmental agent with the pathology of this disease. Several web-enabled databases were mined using appropriate lists of key words covering both subjects, and the gathered information was subsequently filtered and clustered to identify critical genes for which transcription regulation induced by DDT exposure, could happen in an analogous manner to diabetes. Among the most recognized genes that showed preference for the interaction DDT/Diabetes were PPARgamma/PPARalpha, AP-1, DNM1, CAR, NQO1, and MDR1. Real-time PCR analysis of several of these genes were performed on livers of mice injected intraperitoneally with DDT, at a dose and exposure time that did not elicit liver damage (50 mg/kg, 24 h), measured as plasma ALT activity, and histopathological changes. Although it was observed that in DDT-exposed animals, widely known genes, such as PPAR and NQO1, presented a expression profile typical of that found in diabetes; a gene such as FABP5, not previously reported for DDT exposure, but involved in regulation of fatty acid fluxes, could also be used as biomarker of cross-talking between these signaling pathways. These results suggest that beyond epidemiological data, there is increasing molecular indication that DDT could in fact emulate different processes leading to diabetes. Colciencias-UdeC (Colombia), Grant 110745921616.

1900 CONSTRUCTION OF A PBPK/PD MODEL WITH THE EXPOSURE-RELATED DOSE ESTIMATING MODEL (ERDEM) FOR THE PESTICIDE METHAMIDOPHOS IN THE RAT AND HUMAN.

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Methamidophos (O,S-dimethyl phosphoramidothioate) is an organophosphate (OP) insecticide used in agriculture. Its application can result in exposures to workers and the general population. Methamidophos exerts its toxic effect by inhibiting the enzyme acetylcholinesterase (AChE). To better understand the relationship between exposure and tissue dosimetry and AChE inhibition, a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model was constructed in rats and humans. Experimental data obtained from the available literature were used to optimize the model parameters. The PBPK/PD model structure included various physiological tissues/organs and a full gastrointestinal compartment. Tissue-blood partition coefficients were predicted by a computational model. Michaelis-Menten kinetic parameters were used to describe metabolism in the liver and urinary excretion. Bimolecular rate constant was used to describe AChE inhibition. The ERDEM simulation results showed the model could simulate the available experimental data with satisfaction. Therefore, the PBPK/PD model constructed using the ERDEM platform may be helpful in human risk assessment for methamidophos exposure or cumulative risk assessment for OPs with the choice of methamidophos as the index chemical.

1901 DEVELOPING METHODS USING TOXCAST DATA FOR THE CLASSIFICATION AND PRIORITIZATION OF ANTIMICROBIALS AND INERTS.


Improved chemical risk management and increased efficiency of chemical prioritization, classification and assessment are major goals within EPA. Towards achieving these goals, EPA’s ToxCast™ research program has been designed to rapidly screen hundreds to thousands of chemicals’ potential toxicity. In ToxCast, both antimicrobials and inert ingredients are being tested in high-throughput screening systems,
22 in Phase I. Antimicrobial pesticides are chemicals designed to kill or suppress the growth of harmful microorganisms in a variety of use settings, including inanimate objects and surfaces. In total, there are over 300 antimicrobial pesticide active ingredients. Roughly 100 antimicrobial pesticides have undergone re-registration via 41 REDs (re-registration eligibility decision documents), leaving over 200 antimicrobial pesticides requiring some form of hazard evaluation that could be provided by ToxCast. Inert ingredients are substances that are not active ingredients, but which are intentionally included in pesticide products. Limited toxicity data exists for thousands of inert (other) ingredients, creating a need to efficiently determine the potential toxicity of these chemicals. Through the use of ToxCast, toxicity potential has been modeled based on biological activity, pathway-based effects, and estimated dosimetry with a special focus on systemic, cancer, reproductive, and developmental effects. These predictive toxicity scores can then be considered and integrated into the decision process, based on the specific needs of the chemical programs, for classifying antimicrobials and inertes and prioritizing further toxicity testing. This work does not necessarily reflect official Agency policy.

1902 ORGANOPHOSPHATE PESTICIDE POISONING, EXPOSURE SURVEILLANCE SYSTEMS, AND REGULATORY ACTIONS.

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We evaluated data on organophosphate (OP) exposure incidents with a focus on acute poisoning that accounts for significant morbidity and mortality. The elements covered: major U.S. state and national pesticide exposure surveillance systems; reports on and analyses of pesticide exposure incidents; state cholinesterase monitoring programs for workers; pest eradication programs; occupational exposures; and specific cases involving illegal use or misuse; and pesticide use in schools. We examined the U.S. regulatory framework for OPs. Aspects covered included: the 1996 Food Quality Protection Act (FQPA) which requires consideration of the 1996 Food Quality Protection Act (FQPA) regulatory actions resulting from the risk assessment; the integration of toxicology, risk assessment, and risk management functions to minimize human exposure to OPs; and public health significance of the above activities. Results show reduced OP exposure incidents following mitigation efforts by U.S. EPA, and demonstrate the importance of the federal actions necessary.

1903 EFFECT OF OXYFLUORFEN ON MEDAKA (ORYZIAS LATIPES) DEVELOPMENT.

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The productivity of commercial farming for vegetable, fruit, textile, and ornamental crops improves with the use of herbicides to reduce the impact weeds and other plant pests may exert, impeding growth and yield capacity. However, the environmental safety of herbicides (and other pesticides) as related to animals and human, should be established and readily available. Oxyfluorfen (OXY), 2-chloro-1-(3-ethoxy-4-nitrophenyl)-4-(trifluoromethyl) benzene, a persistent polychlorinated dipheny ether (PCDE), is applied to many food crops targeting weeds on contact. With a low solubility, OXY is stable, sorbs to sediments and toxic to aquatic fish. We used the fish model, Japanese medaka, to validate the toxicity of OXY, proposing to establish the effects on early life of medaka by identifying LC50, hatching efficiency, post hatch growth, and skeletal development. Medaka embryos (21 hours post fertilization, hpf) and hatchlings (1 day post hatch, dph) were exposed to OXY (0.5 ppm-8 ppm) in 96 h static renewal replicates. Our results suggest a low chorioid permeability, revealing no LC50, and no change in hatching duration. However, we observed cardiovascular developmental deformities in higher OXY concentrations (4-8 ppm), indicating OXY crossed the chorion. Treatment of hatchlings revealed a LC50 of ~3 ppm. We also observed socalosis and alimentary canal hemorrhaging in hatchlings after 96 h exposure of 4 ppm-8 ppm, and vertebral body irregularity in hatching exposed to 2 ppm. After 7 days depuration, 96 h exposed fish stained and measured, revealed a shorter neurocranium than control. Such findings support further study of OXY on medaka development.

1904 TOXICOLOGICAL ASSESSMENT OF INITIUM®

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Initium® (proposed common name: Ametoctradin; IUPAC: 5-ethyl-6-octyl[1,2,4] triazolo[1,5-a] pyrimidin-7-amine; BASF internal code: BAS 650 F) belongs to the new chemical class of pyrimidylamines developed by BASE. Initium® is a potent inhibitor of complex III, an enzyme of the mitochondrial respiratory chain of oomycte pathogens. Initium® will be used as a new contact fungicide active ingredient with long-lasting preventive action in various specialty crops. An overview of the excellent toxicological profile of Initium® is provided below. Initium® is of low acute toxicity (rat LD50 (oral): >2000 mg/kg bw, rat LD50 (dermal): >2000 mg/kg bw, rat LC50 (inhhalation): > 5.5 mg/L), is not a skin or eye irritant and no skin sensitiser. No target of toxicity could be identified in short-term or chronic toxicity studies; the NOAELs obtained in 90-day feeding studies in rats, mice and dogs exceeded the limit dose level of 1000 mg/kg bw/day. Initium® displays mutagenic effects in the Ames test, and in vitro and in vivo tests for carcinogenicity and reproductive toxicity were negative. The definition addresses public and environmental health effects, as well as exposure, and demonstrates the importance of the federal actions necessary.

1905 EFFECTS OF PESTICIDES ON AROMATASE EXPRESSION IN BIOULUMINESCENT MICE AND GSK-3BETA/BETA-CATENIN SIGNALLING IN LINCAP HUMAN PROSTATE CANCER CELLS.

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Certain pesticides are known to disrupt the endocrine system in humans and wildlife and are suspected of causing endocrine-related diseases, such as reduced fertility, impaired fetal/child development and hormone-dependent cancers of the breast, ovary, testis and prostate. Several hormone-dependent cancers are associated with dysfunction of aromatase (CYP19), the enzyme responsible for converting androgens to estrogens; its inhibition can lead to infertility and osteoporosis in women, and decreased sperm production in men. Overexpression of aromatase is associated with pro-proliferative effects, most notably in breast cancer. It is also suggested that hormone-independent mechanisms, such as the GSK-3beta/beta-catenin pathway are involved in the development/progression of certain cancers, such as prostate cancer. In vitro studies suggest that atrazine induces aromatase, but little evidence exists in vivo. We studied the effects of atrazine in a bioluminescent Cyp19-luciferase transgenic mouse model (Caliper LifeSciences; line 125), which expresses luciferase under control of the gonadal PII Cyp19-promoter; the mice can be scanned in real-time without need for sacrifice. In males, forskolin (10 mg/kg, ip, single injection), a potent inducer of PII-mediated aromatase in vitro, increased bioluminescence 3-5 days after exposure, but only in a few individual mice. Atrazine (100 mg/kg, once, or 30 mg/kg, daily for 5 days) did not increase bioluminescence for up to 7 days after initial exposure. Ex vivo tissue analysis (testis, epididymis) found a statistically significant increase in bioluminescence in forskolin-, but not atrazine-treated mice. In our hormone-dependent LNCap prostate cancer model, >1 μM of vinclozolin inhibited nuclear androgen receptor and beta-catenin accumulation in the presence of 10 nM DHT. Together, these findings suggest atrazine may not be an effective gonadal aromatase inducer in vivo and that vinclozolin may act as antiandrogen via AR-dependent and -independent mechanisms.

1906 A METHOD FOR CALCULATING CUMULATIVE IMPACTS.

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The California Environmental Protection Agency has adopted a working definition of cumulative impacts from environmental pollutants that is geospatially based. The definition addresses public and environmental health effects, as well as exposures, while considering socioeconomic factors and sensitive individuals. From this definition we are developing a method to assess cumulative impacts for the purpose...
of prioritizing areas to target for reducing pollution burden. Three broad components of impact are assessed: exposures, public health effects and environmental effects. While specific exposure and toxicity data are emphasized, other information characterizing the community exposure is considered. Exposure indicators include reported emissions, monitoring data, and other known or likely exposures. Public health effects include health outcomes that have been linked to chemical exposures, such as asthma, low birth weight and lung cancer. Environmental effects reflect the conditions of the geographic area, such as ecological degradation. Each component is considered from a multimedia perspective, and the method takes into account sensitive populations and socio-economic factors as a modifying function. Data for each component are obtained for the specified geographic area from publicly available databases and government sources. The size of the geographic area is case-specific, but can accommodate a range of data sources. The demographic data is community specific with emphasis on those factors that influence toxicity and health effects. Since the data are drawn from multiple sources and reflect different types of information, the results are considered semi-quantitative and are best represented as generalized groupings such as low, medium, and high. While the choice of information and method of analysis is best decided following a scoping of the problem with interested stakeholders, an example comparing 25 communities is presented.

**1907 THE NRC REPORT ON PHTHALATES AND CUMULATIVE RISK ASSESSMENT: FOCUS ON CUMULATIVE RISK AND COMMON ADVERSE OUTCOMES.**

D. A. Cory-Slechta. Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY. Sponsor: D. Cory-Slechta.

This NRC Committee was asked to consider whether cumulative risk should be considered for phthalates, and to consider cumulative risk assessment approaches more broadly. In addition to a positive response for consideration of cumulative risks arising from phthalates, the Committee recommended two strategies to significantly enhance cumulative risk assessment broadly. The first was to move cumulative risk from a focus on structurally and mechanistically-related chemicals, the current basis for cumulative risk, to a strategy that focuses on cumulative risks from chemicals that produce common adverse outcomes, i.e., shared physiological consequences as per the common adverse effect of phthalates on male reproductive dysfunction. Since this dysfunction arises from androgen insufficiency, the Committee recommended that in a cumulative risk assessment for phthalates, other chemical and non-chemical factors that likewise lead to androgen insufficiency, regardless of the mechanism by which they produce androgen insufficiency, should be considered cumulatively with phthalates. Secondly, the Committee recommended that this strategy should be applied to other common adverse outcomes, e.g., studying the cumulative impact of chemicals in combination, e.g., lead, methylmercury, PCBs, that have individually been shown to result in IQ reduction. A focus on common adverse outcomes should actually facilitate and expedite the shift to cumulative risk assessment because the approach defines the groups of agents that should be considered in combination for a common adverse outcome, thereby defining and circumscribing the mixture problem in the context of health effects.

**1908 PROPOSED MODE-OF-ACTION FOR PHTHALATE-INDUCED MALE REPRODUCTIVE EFFECTS: CONTRIBUTIONS OF DIET-INDUCED OBESITY AND PHTHALATE EXPOSURE.**


A recent National Academies of Sciences (NAS) report urged the U.S. EPA to develop a cumulative risk assessment for phthalates. The NAS recommended that phthalates and other agents that cause androgen insufficiency or block androgen receptor signaling should be included in a cumulative risk assessment based on common adverse outcomes and not focus exclusively on structural similarity or similar mechanisms of action. Based on the recommendations of this report, the U.S. EPA's Integrated Risk Information System (IRIS) is developing an IRIS human health assessment for the phthalates. This assessment will include phthalates which elicit the phthalate syndrome (infertility, decreased sperm concentrations, and other reproductive tract malformations). Other nonchemical stressors, such as diet, may contribute to phthalate induced effects in developing and adult males. Exposure to high-fat diets can promote metabolic syndrome which has been shown to have a negative effect on male reproductive health by reducing testicular testosterone production, and plasma testosterone. In vivo and cell culture studies suggest that adipocyte-secreted cytokines can reduce Leydig cell function, testosterone, and spermatogenesis. Data suggest that phthalate exposure may also promote obesity. Furthermore, various in vitro studies suggest that phthalate exposure promotes adipogenesis through activation of the peroxisome proliferator activated receptor gamma in preadipocytes. When combined, exposure to both a high-fat diet and phthalates could potentially induce obesity and exacerbate phthalate induced effects on the male reproductive system. The proposed mode of action for phthalate mechanism by which a nonchemical stressor (nutrition) may contribute to the cumulative effects of phthalate exposure. These authors' views do not necessarily reflect the views or policies of the U.S. EPA.

**1909 CRITICAL EVALUATION OF THE DATA UNDERLYING THE USA TODAY RANKINGS OF AIR QUALITY AT SCHOOLS.**

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In December of 2008, the USA Today released "The Smokestack Effect," a report that ranked air quality at US schools based on a calculated score developed from modeled airborne concentrations of various chemicals that were developed using EPA Toxic Release Inventory data and RSEI model. In addition to a percentile ranking, the USA Today report also identified the chemicals that contributed most significantly at each school, as well as the companies responsible for emitting those chemicals. However, the report did not provide any information regarding the modeled airborne concentrations used to develop the rankings. This information is critical for determining whether any of the chemicals may be present at concentrations that could pose a health threat. In order to address this, we obtained all of the underlying data and conducted a comprehensive analysis, focusing specifically on schools ranked in the first and second percentiles. Modeled airborne concentrations were then compared to health-based benchmarks, as well as to available monitoring data. We found that the modeled air concentrations were generally higher, but comparable to measured levels. Additionally, for the majority of the schools that were predicted to be the most highly impacted based on the USA Today rankings, monitoring concentrations were generally below health-based screening levels, indicating that the USA Today rankings are not a reliable indicator of health risk. These findings are further supported by air monitoring data, recently collected by EPA at many of the schools identified as impacted by USA Today study, showing measured concentrations below health benchmarks. This is an important finding given the panic that erupted in communities across the country following release of the USA Today study.

**1910 AGE GROUPINGS FOR APPLICATION OF AGE-SENSITIVITY FACTORS IN ASSESSING RISK FROM CARCINOGEN EXPOSURE EARLY IN LIFE.**

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California’s Air Toxics Hot Spots program requires risk assessment of stationary sources of air pollutants to be conducted in accordance with guidelines produced by the Office of Environmental Health Hazard Assessment. These guidelines were recently updated to include more explicit consideration of exposures that occur early in life. Analyses conducted by the Office of Environmental Health Hazard Assessment indicated that for most carcinogens postnatal exposures in rodents resulted in higher potency estimates. This analysis resulted in identifying default factors to apply to potency estimates for early life exposure. Based on this analysis and that conducted by U.S. EPA in 2005, OEHHA’s guidance requires application of a 10 fold Age-Sensitivity Factor (ASF) when estimating risk from exposures occurring in the third trimester up to 2 years of age, and an ASF of 3 for exposures occurring from age 2 up to age 16 years. This is similar to guidance by the U.S. EPA with some notable exceptions. We require application of the ASFs to all carcinogens, regardless of purported mode of action unless there is evidence to the contrary. Further we include exposures in the third trimester in our estimates of risk. Our choice of the age bins is based on information regarding differences in toxicokinetics and toxicodynamics between infants, older children and adults. This paper will discuss our analysis of appropriate age groupings for application of these ASFs and our rationale to apply these factors to all carcinogens.

**1911 CONSIDERATION OF CHILDREN’S RISK: METHODS AND ADEQUACY OF UNCERTAINTY FACTORS.**

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Several organizations (e.g., WHO, U.S. EPA) have developed frameworks for evaluation of issues related to children’s risk, noting the importance of consideration of differences between adults and children in developmental processes, the resulting...
kinetic and dynamic differences, and the implications for tissue dose and response. This work reviews key information on kinetic differences between adults and children and the resulting implications for tissue dose, along with issues of data interpretation, noting that uncertainty in dose metrics or models for the pediatric population in result in different conclusions regarding the adequacy of the default human variability uncertainty factor. Analyses of the need for a developmental neurotoxicity study will be critically reviewed, and discussed in the context of the choice of the database uncertainty factor. Analyses of the data obtained from various study types and implications for the database uncertainty factor are also discussed. For example, if a rat chronic and reproductive NOAEL are available, a database factor of 3 is sufficient to cover the 95th percentile of the chemicals evaluated. Implications of biological differences are discussed, along with critical data gaps and uncertainties, and the risk assessment implications of these issues are considered.

1912 USE OF LIFE-STAGE ADJUSTMENT IN ORAL RISK ASSESSMENT FOR O-TOLUIDINE.


Since o-toluidine is used in the formulation of some elastomers, coatings or sealants that contact drinking water, NSF/ANSI 61 Annex A (2008) and U.S. EPA (2005) risk assessment guidelines were used to determine an acceptable level of o-toluidine in drinking water. Occupational exposure to o-toluidine has been associated with an increased risk of bladder cancer. The weight of evidence suggests that it is genotoxic in vitro and in vivo. Chronic dietary exposure to o-toluidine hydrochloride in F344 rats was associated with sarcomas of multiple organs in each sex, fibromas of the subcutaneous tissue and mesotheliomas in multiple organs or the tunica vaginalis in male rats, and sarcomas of the spleen, urinary bladder transitional-cell carcinomas, and mammary gland fibroadenomas or adenomas in female rats. Chronic dietary exposure in B6C3F1 mice was associated with histiocytic and cellular tumors in both sexes in both the life stages and at different exposure levels. The relationship of TD, the relationship of animal:human doses producing the same TTC, UFH-TK is the ratio of animal:human doses producing the same TTC, UFH-TK is the ratio of TTC, generally representative of toxicokinetic and toxicodynamic data for o-toluidine during early life exposure. The life-stage adjusted 10% cancer risk level of 0.00047 mg/kg-day corresponds to a l

1913 DATA-DERIVED EXTRAPOLATION FACTORS FOR INTER- AND INTRASPECIES EXTRAPOLATION.


Two key tenets of toxicology are 1) toxicity at the target tissue causes the response and 2) dose defines the response, representing toxicokinetics (TK) and toxicodynamics (TD), respectively. Effective risk assessment requires clear presentation and evaluation of variability, with special emphasis on quantifying uncertainties or models for the an-exposure (RIV) derivation, replacing default uncertainty factors (UF) with target tissue concentration (TTC) data and differences in TTC at predetermined levels of risk is key to reducing uncertainty in inter- and intraspecies extrapolation: UFA and UFH, respectively. Dividing these UF’s into TK and TD components facilitates their representation with data and extrapolation (DDEF). UFA-TK is the ratio of animal:human doses producing the same TTC, UHF-TK is the ratio of TTC at the same dose in sensitive:generally-representative humans. DDEFs are used data from the toxicologically active chemical form near the point-of-departure in the relevant species and population group. The linearity of the relationship between exposure and TTC should be determined. DDEF values may not be consistent for all effects, so DDEFs and RIVs for several effects should be developed. This approach is described in a U.S. EPA draft document undergoing external peer review. EPA expects this document to stimulate targeted research and ultimately reduce the uncertainties in health risk assessment. May not reflect EPA policy.

1914 EVALUATION OF THE MAGNITUDE OF TOXICOKINETIC INTER-INDIVIDUAL VARIABILITY FACTOR (IVF-TK): IMPACT OF SUBPOPULATIONS AND CHEMICAL CHARACTERISTICS.

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The IVF-TK used in non-cancer risk assessment corresponds to a default value of 3.16. The objective of this work was to evaluate the magnitude of IVF-TK as a function of physico-chemical/biochemical properties and pathway-specific rates of metabolism of toxics in various subpopulations. A steady-state algorithm (SSA) was used to compute the internal dose (blood concentration (Cblood) and rate of metabolite produced/L liver (RAM)) of surrogate chemicals metabolized by ADH, CYP2E1, CYP1A2 and CYP3A4 in four human subpopulations (neonates, adults, elderly, pregnant women). In order to solve the SSA, data on body weight and height, hepatic content of CYPs and ADH, liver blood flows and volumes, renal function and alveolar ventilation rates were obtained from the literature or PPM software. In the case of CYPs, the total for human adults (C) was within a factor of 2 of published experimental values for drugs and VOCs. Using Monte Carlo simulations, IVF-TK (as the ratio of the 95th percentile value for each subpopulation over the 50th percentile value in adults) was computed for Cblood and RAM for various blood-air partition coefficient (Pb) and hepatic extraction ratio in adults (E). For oral exposure, Cblood-based IVFs of 3.16 in case of CYP1A2 substrates in neonates (max.: 0.2) when E = 0.1 – 0.3 and Pb ≥ 1000 or when E = 0.5 – 0.7 and Pb ≥ 100. For inhalation exposure, Cblood-based IVF-TKs ≥ 3.16 was observed for CYP2E1 substrates in neonates (max.: 5.8) when E = 0.01 – 0.2 and Pb ≥ 1000 or when E = 0.3 – 0.7 and Pb ≥ 100. Comparable results were obtained for CYP3A4, CYP1A2 and ADH pathways, with higher values for CYP1A2 (max.: 12.4). In subpopulations other than neonates, the default IVF-TK value was never exceeded based on Cblood. The IVF-TK, based on RAM, was below 3.16 in all cases. Overall, this study has identified chemicals and subpopulations for which the default IVF-TK is (not) likely to be protective, based on the consideration of critical determinants of the relevant processes.

1915 RISK ASSESSMENT STRATEGIES FOR PROTECTING CHILDREN’S HEALTH.

A. G. Salmon, M. A. Marty, B. Winder, K. Riveles and J. P. Brown. OEHHAA, CalEPA, Oakland, CA.

Various studies of the health effects of toxic chemicals have suggested that the nature and severity of effects can vary depending on the age at which the exposure occurs. In particular, infants and children are often more sensitive than adults. It is therefore a particular concern of risk assessment to include consideration of special impacts on infants and children when estimating health protective exposure levels or risk factors for the general population. In response to a State of California legislative mandate, OEHHAA has over the past several years studied strategies for including these considerations in risk assessments for air toxics. Where compound-specific data on age-dependent differences in sensitivity exist, these would naturally be included in the risk assessment. However in most cases the only toxicity data (either human or animal) are for exposures to adults. In this case it is necessary to extrapolate to effects on infants and children. As a result of this concern, OEHHA initially developed a prioritization of individual chemicals, which found that toxicity to certain organ systems (e.g. the nervous and respiratory systems) or certain processes (e.g. carcinogenesis) showed greater potential for differential impacts on the health of infants and children. Subsequently, OEHHAA has published risk assessment guidelines which recommend specific measures to take account of this differential impact. For non-cancer effects changes in the methodology for individual chemical assessments were prescribed, including an increased uncertainty factor for interindividual variability. This is in contrast to the cancer risk assessment guidelines which apply overall adjustment factors for early-in-life exposures to the existing (adult) potency values. We will present detailed rationales for these changes and illustrations of their numerical impact for specific non-cancer risk assessments. For instance, recently published RELs for manganese and acrolein included factors of 10 and 3 respectively to allow for differential impacts on children.

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The Chemical Security and Analysis Center (CSAC), a U.S. Department of Homeland Security component, is responsible for homeland security needs related to chemical threat awareness. The CSAC has been charged to determine public health impacts (injuries and deaths) potentially associated with catastrophic chemical releases. The following methodology was developed to address this need, and applied to 17 chemicals to date. Injury-severity categories were defined based on expected duration, permanency and extent of potential injuries for acute exposures. These categories include ‘mild injuries,’ ‘incapacitating injuries,’ ‘life-threatening injuries,’ and ‘death.’ As described below, probit curves were developed for each chemical, exposure route (inhalation, ingestion, dermal contact), and injury-severity category. First, toxicity endpoints were defined for each exposure route and injury-severity category through a literature review. Next, dose-response (esp. incidence) data from key studies were evaluated, and allometric equations were used when necessary to derive human-equivalent exposure estimates from the results of animal studies. Median effective and lethal exposures were calculated for each exposure route and injury-severity category based on probit analyses of the transformed data. Alternative methods were developed to derive predictive toxicity measures when the data from empirical investigations were inadequate (e.g., route-to-route, severity-category-to-severity-category, and chemical-to-chemical extrapolations). In addition, methods were developed to assign uncertainty factors to each predictive measure, enabling the depiction of a bounding range that reflects the uncertainty associated with each measure. The resultant predictive measures serve as toxicological inputs to the CSAC’s Chemical Terrorism Risk Assessment, and represent a more refined way to assess and incorporate non-lethal effects into other risk assessments.

1918 A Method for Selecting Toxicity Reference Values from Multiple Sources for Human Health Risk Assessments.


The decision to use a particular toxicity reference value (TRV) in human health risk assessment (HHRA) can be complicated when considering the multiple sources that are available, as well as the public health and regulatory implications that might arise from using one value over another. The process of selecting a TRV for a chemical can depend on the preference or recommended practice of the local or regional health authority, the recommendations of other jurisdictional organizations, or the latest science in the peer-reviewed literature. Potential methods of selecting TRVs may range from selecting the most defensible value, to the use of a pre-defined hierarchy, or simply, using the most conservative available value. In some instances, decisions made on process rather than science may be made, and these can have a notable impact on the results of an HHRA. Additionally, the process used to select TRVs may impact mixture toxicity assessment as substances can be assigned to ‘mixtures’ based upon the toxicological endpoint(s) associated with the TRV used. In some instances, these endpoints may be different than the generally understood health effects for a given substance. This presentation will provide a description of methods that are used in Alberta, Canada to select TRVs for environmental impact assessments in the areas of oil, gas, mining, energy, and other industrial developments, and will also identify some pros and cons of various methods.

1919 Development and Application of a Risk Assessment Paradigm to Acetaldehyde: A Tobacco Smoke Constituent.


The scientific literature detailing the identity of tobacco smoke constituents potentially responsible for the adverse health effects of cigarette smoking has grown significantly over the last 20 years. Increasingly there is a trend towards providing a quantitative risk estimate of the contribution of individual constituents with the aim of prioritising tobacco smoke constituents for further research. Our assessment methodology uses the margins of exposure (MOE) model to indicate whether a smoke constituent is a high priority for exposure reduction research. The MOE model developed by the European Food Safety Authority (EFSA) and acknowledged by the European Union (EU) scientific committees has then been applied to the data. To do this, the literature was first reviewed for relevant experimental and/or epidemiological data to compute a benchmark dose (BMDL10) based on diseases considered most representative of tobacco smoke exposure: lung cancer and chronic obstructive pulmonary disease (COPD). Furthermore, to indicate the strength of the data, a series of MOE values from the range of published studies has been computed to derive a MOE range. MOE values <10,000 indicate a high priority for exposure reduction research. For acetaldehyde, from one publication, using an acetaldehyde smoke yield of 1448 µg per 114F cigarette, four MOEs are generated from two carcinogenic endpoints in two genders. The MOE values generated are 178, 308, 805 and 988 indicating that this constituent would be identified as being of high priority. The methodology shown here has been identified as a useful tool which may be used for initial risk assessment prioritisation of tobacco smoke constituents. However, it must be noted that the MOE approach is only one segment of the paradigm which needs to be undertaken to ascertain the individual toxicants importance to smoking-related diseases.

1920 Analysis and Comparison of Bladder Tumor Induction by Biphenyl, Saccharin, and Melamine.


The current Integrated Risk Information System (IRIS) database entry for biphenyl presents a cancer weight-of-evidence classification of ‘not classifiable as to human carcinogenicity.’ Recent carcinogenicity studies of biphenyl (Umeda et al., 2005, 2002) provide new data to reevaluate the cancer weight-of-evidence classification. In a 2-year carcinogenicity bioassay in male and female F344 rats (Umeda et al., 2002), administration of 4500 ppm biphenyl in the diet induced bladder tumors (transitional cell papillomas and carcinomas) in males but not females. The occurrence of bladder tumors was closely related to the formation of urinary bladder calculi composed of 4-hydroxybiphenyl-O-sulphate in male rats exposed to high doses of biphenyl. The high pH and cation level in male rat urine were critical for precipitation/calcification formation in the bladder. Transitional epithelial hyperplasia was observed in all male rats that developed bladder tumors. Bladder calcification and regenerative hyperplasia of transitional epithelial cells were dose-dependent, as demonstrated by the absence in low-dose rats. Similarly, studies have shown that high-level exposure to saccharin and melamine induced bladder tumors in male rats. The bladder tumors were preceded by urine precipitation/calcification formation and hyperplasia. A comparison of the endpoints related to urinary precipitation/calcification and bladder tumor formation after exposure to different doses of
biphenyl, saccharin and melamine was conducted. These endpoints included: physiologic parameters of the urine (pH, cation level, osmolality), ulceration and inflammation in the bladder, transitional epithelium hyperplasia and bladder tumor occurrence. This cross chemical review of bladder tumor data can inform the cancer mode of action for biphenyl. [Disclaimer: The findings and conclusions in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

1921 A CRITICAL REVIEW OF THE EFFECTIVENESS OF PHARMACEUTICAL CARCINOGENESIS TESTING.

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The objective of this exercise was to determine the current effectiveness of nonclinical testing to predict carcinogenicity risk for humans. The electronic version of the Physician’s Desk Reference (PDR) was mined for data pertaining to carcinogenicity testing results in rodent models and human experience. Analyses of the compounds using this database showed that there were 36 drugs with significant concern of cancer in humans. These drugs were classified into the following three categories: (1) hormones, (2) immunosuppressives, and (3) cancer chemotherapeutics (predominantly genotoxic). Of these 36 compounds, 16 were positive for carcinogenicity in rodents, 6 were negative in rodents, and 14 were not tested in rodents. There were 207 compounds with positive carcinogenic results in rodents but only 36 with human concern evidenced by statements in precautions or warnings in the drug label suggesting a potential significant false positive rate of over 80%. The generally recognized human pharmaceutical carcinogenicity risk factors of hormonal modulation, immunosuppression, genotoxicity and chronic tissue injury do not require 2 year bioassays in rodents for identification. Furthermore, immunosuppressive drugs typically tested negative in rodent carcinogenicity assays. Results suggest that some improvement may be possible in testing strategies beyond those in standard practice today.

1922 ESTIMATING THE FREQUENCY OF HORMESIS IN THE AMES ASSAY.

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This study assessed the occurrence of hormetic dose responses from a previously published data set of 4,335 dose-response assays. These assays were designed to assess the mutagenicity of 270 chemicals in five tester strains of the Ames Assay (with and without S9 fraction), in a five dose protocol involving semi-log spaced doses. Assays that were considered for analysis were selected based on an a priori entry criteria that required an assay to have: (1) a derivable benchmark dose, (2) three doses below the BMD10, (3) minimal variation, and (4) statistical evidence of a mutagenic response at the highest dose. There were thirty-three dose responses that satisfied this a priori entry criteria. Of that total, 19 (57.5%) dose-responses demonstrated statistically significant evidence of hormesis for base pair substitution and frameshift mutation. This increase in dose-responses satisfying the evaluative criteria for hormesis exceeded by 4-fold that which would have been predicted to occur by chance alone (p<0.001). After adjusting for the occurrence of false positive and negative responses, the frequency of hormesis approached 100.0 %. These findings challenge the current linearity at low-dose paradigm that is used as a default assumption by regulatory agencies when evaluating mutagenicity.

1923 TOXICOGENOMICS IN RISK ASSESSMENT: APPLICATIONS AND CHALLENGES.

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National Research Council’s report on Toxicity Testing in the 21st Century envision greatly expanded use of toxicity pathways and the systems biology approach in the implication of human health risk assessment. There is high expectation for the science of toxicogenomics to decrease the uncertainties associated with the risk assessment process by providing valuable insights into mode of action and dose response. Thus far, one of the most promising applications of toxicogenomics has been in screening and prioritization of drug development. In addition, toxicogenomics has been used to understand specific pathways and chemical signatures of toxicity and identification of biomarkers. Furthermore, well-defined toxicogenomics studies have been used to distinguish genotoxic from non-genotoxic compounds (Mutat Res. 2005, 575:561-84), and in dose-response analysis (Toxicol Sci., 2008, 105:368-83; Toxicol Sci. 2007, 98:240-8). Specific case studies have also been conducted with the intention of incorporating toxicogenomics in risk assessment (EPA, 2009). Identification of proper mechanism of action can lead to increased confidence and consistency in risk assessment practices. However, the use of toxicogenomics is not without challenges. Well-defined experimental designs, selection of proper endpoints, selection of dose ranges and time points, validation and interpretation of toxicogenomics data, availability of proper statistical models, and development of quantitative models capable of accurately predicting the data are a few of the challenges. The most significant challenge is integration of toxicogenomics data with available toxicity data in order to reduce uncertainties and support mechanistically-based quantitative risk assessment. In spite of these challenges, toxicogenomics is expected to play an increasingly larger role in regulatory decision making. (The views expressed are those of the author and do not necessarily reflect the views or policies of the U.S. EPA).

1924 EVALUATING THE ROLE OF α2u-GLOBULIN ACCUMULATION IN THE RENAL CARCINOGENICITY AND TOXICITY OF HEXACHLOROETHANE (HCE).


Hexachloroethane (HCE) is a volatile solid used in smoke pots, smoke grenades, and pyrotechnic devices and has been used as a polymer additive, moth repellant, plasticizer for cellulose esters, insecticide solvent, and in metallurgy for refining aluminum alloys. Oral exposure to HCE in rodent models leads to renal toxicity (e.g., tubular nephropathy, tubular nephrosis, increased absolute and relative kidney weight, hyaline droplet accumulation, tubule regeneration, granular casts, and increased proliferation) and renal carcinogenicity (e.g., tubule adenomas and carcinomas). The occurrence of renal tumors and tubular nephropathy in male rats suggests potential involvement of male rat-specific α2u-globulin mode of action. In developing the human health risk assessment of HCE for the EPA’s Integrated Risk Information System, the HCE database was evaluated to determine if α2u-globulin plays a role in the development of renal tumors and toxicity. Although the available HCE data indicate renal carcinogenicity and toxic effects similar to the characteristic renal effects induced by the α2u-globulin mode of action, immunohistochemistry confirmation of the α2u-globulin protein in the hyaline droplets of renal tubules has not been performed. Furthermore, kidney effects were observed in both HCE exposed male and female mice, and female rats, none of which generally accumulate low molecular weight protein in the kidney. These data suggest a mode of action other than α2u-globulin-related effects.

1925 CHALLENGES IN THE IRIS HEALTH ASSESSMENT OF HALOGENATED PLATINUM SALTS AND PLATINUM COMPOUNDS.


The U.S. EPA’s Integrated Risk Information System (IRIS) Program is developing a health assessment for halogenated platinum (Pt) salts and Pt compounds because the general population may be exposed to Pt and there is a well-established relationship between occupational exposure to halogenated Pt salts and Pt-specific allergic sensitization. Exhaust from catalytic converters is the major source of environmental Pt which contains predominately water-insoluble metallic or oxide forms of Pt. Although insoluble forms of Pt are unlikely to be associated with allergic sensitization, environmental transformation can potentially transform insoluble Pt to water-soluble forms. There are multiple sources of uncertainty in assessing the health effects associated with chronic exposure to Pt including human variation in susceptibility, extrapolation from studies of less than chronic exposure duration, and deficiencies in the Pt toxicity database. Additional sources of uncertainty include: (1) variations in exposure measurements of Pt compounds associated with Pt-specific allergic sensitization in the occupational literature, (2) extrapolation of exposure measurements of soluble Pt compounds to individual halogenated Pt salts, (3) challenges of evaluating sensitization from a group of Pt compounds, (4) use of...
a hexachloroplatinitic acid skin prick test as a measure of Pt-specific allergic sensitization, and (5) consideration of smoking as a risk factor for Pt-specific allergic sensitization. Given that the concentration of Pt in ambient air has risen since the introduction of the catalytic converter and use of Pt as a diesel fuel additive, these sources of uncertainty are particularly important to an evaluation of the health effects of halogenated Pt salts and Pt compounds. [The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

And, efforts to develop BBDR models for risk assessment should consider resource and time requirements relative to their potential benefits. Disclaimer: Views expressed in this poster represent those of the authors and do not reflect the views or policies of the U.S. EPA.

1926 SKIN SENSITIZATION: THE COLIPA STRATEGY FOR DEVELOPING AND EVALUATING NON-ANIMAL TEST METHODS FOR RISK ASSESSMENT.


The sensitizing potential of chemicals is usually identified via animal studies, such as the local lymph node assay. Due to the increasing public and political concerns regarding the use of animal for the screening of new chemicals, the Colipa Skin Tolerance Task Force collaborates with and/or funds academic research groups to increase and apply our understanding of the molecular and cellular events occurring during the acquisition of skin sensitization. Fundamental and applied research is being funded in the following key areas: chemistry/peptide binding, skin metabolism, skin bioavailability, evaluation of biomarkers for DC activation and T cell proliferation. Knowledge gained from this research is being used to support the development and evaluation of novel alternative approaches for the identification and characterization of skin sensitizing chemicals. At present three non-animal test methods [Direct Peptide Reactivity Assay (DPRA), Myeloid U937 Skin Sensitization Test (MUSS) and human Cell Line Activation Test (h-CLAT)] have been evaluated via interlaboratory ring trials for their potential to predict skin sensitization and were recently accepted by the ECVAM for formal pre-validation. Data from all three test methods will be used to support the development of testing strategy approaches for skin sensitizer potency prediction. The replacement of the need for animal testing for skin sensitization risk assessment is viewed as ultimately achievable and the next couple of years should set the timeline for this achievement.

1927 MAJOR CHALLENGES TO BIOLOGICALLY-BASED DOSE-RESPONSE MODELING FOR ESTIMATING LOW-DOSE HUMAN RISK USING MOLECULAR TOXICOLOGY DATA.


The strength of recent advances in molecular toxicology is that they can provide information on more proximal markers of dose and on early markers of contributions from multiple pathways to diseases. Since biologically-based dose response (BBDR) modeling can incorporate data at the cellular and molecular level, some have suggested it can serve as a link between data generated on toxicity pathway perturbations and making estimates of risk for adverse responses at low doses. This poster presents the point of view that there are likely serious impediments to developing BBDR models for this specific purpose. BBDR models have many uses, which include evaluating proposed mechanisms of toxicity, understanding how multiple variables can affect disease processes, and identifying data gaps. However, their application to estimating low-dose human risk (limited so far to cancer clonal growth modeling) has not improved the reliability of risk predictions. This is because BBDR models do not eliminate the need for empirical modeling of the relationship between dose and effect, but only move it from the whole organism to a lower level of biological organization, while introducing significant new sources of uncertainty. Quantitative inferences from these data are limited by inter- and intra-individual heterogeneity that cannot be eliminated with available or anticipated experimental techniques. Also, BBDR modeling does not avoid uncertainties in the mechanisms of toxicity relevant to low-level human exposures. Thus, before a BBDR model is used to set human exposure standards, the robustness of model predictions must be evaluated against the limitations and sources of uncertainties. EPA has recently evaluated the information provided by developmental neurotoxicity studies (DNTs) on pyrethroid pesticides. The Agency has six guideline DNTs available for review; one Type I (bifenthrin), four Type II (beta-cyfluthrin, lambda-cyhalothrin, zeta-cypermethrin, deltamethrin) and one mixed syndrome pyrethroid (fenpropathrin). These studies have been evaluated in the context of: pyrethroid mode of action, in vivo toxicity syndromes typical for Type I and II pyrethroids, pharmacokinetic properties, critical effect(s) selected for risk assessment, and the open scientific literature. Generally, the findings reported in these studies were decreases in pup weight and/or pup weight gain, and/or brain weight decrease. None of the studies show effects related to those which are uniquely studied in the DNT and are intended as measures of impacts on the developing nervous system (e.g., effects on learning, memory, morphometrics, etc.) at doses lower than those eliciting decreases in pup weight, pup weight gain, or brain weight. One study showed an increase in startle response (fenpropathrin). Neurological clinical signs observed in pups included increased grooming counts (bifenthrin) and vocalizations (deltamethrin). None showed tremors or other clinical signs expected for pyrethroids. Critical endpoints for risk assessment were typically selected from acute neurotoxicity studies and/or dog studies, not from the DNT. Only one DNT study (i.e. zeta-cypermethrin) was used to identify a point of departure (PoD) for the short- and intermediate-term incidental oral and dermal endpoint (children only). Although several literature studies have demonstrated juvenile sensitivity to pyrethroids when compared to adults, and the metabolic systems in juveniles are less developed than adults, the pyrethroid DNT data indicates that this study is not particularly useful for identifying this comparative sensitivity. (This abstract does not represent U.S. EPA policy).
In the given document, the authors discuss various aspects of human variability and its impact on risk assessments and exposure modeling. These aspects are crucial in understanding how individuals respond to environmental exposures, which is particularly important given the increasing complexity of environmental contaminants.

### Issues in Using Human Variability Distributions to Estimate Low-Dose Risk

W. A. Chiu, K. S. Crump, and R. Subramanian, U.S. Environmental Protection Agency, Washington, DC, and Louisiana Tech University, Ruston, LA.

**Background:** A committee of the National Academies of Science recommended using human variability distributions (HVDs) to estimate low-dose risks in certain situations. In this approach (HVD modeling), normal distributions are estimated from data on pharmacokinetic and pharmacodynamic variables that impact individual sensitivities to the toxic response. These distributions are combined into an overall log-normal distribution by assuming the variables act independently and multiplicatively. This distribution is centered at a point of departure (PDD) dose usually estimated from animal data. The resulting log-normal distribution is used to quantify low-dose risk.

**Objective:** To examine the implications of various assumptions in HVD modeling. Methods: Assumptions and data used in HVD modeling are subjected to rigorous analysis. Results: The assumption that the variables affecting human sensitivity vary log-normally is not scientifically defensible. Other distributions that are equally consistent with the data provide very different estimates of low-dose risk. HVD modeling often involves assuming that the threshold dose, defined by dichotomizing a continuous apical response, has a log-normal distribution. This assumption is incompatible (except under highly specialized conditions) with assuming that the apical response itself is log-normal. However, the two assumptions can lead to very different estimates of low-dose risk. The assumption in HVD modeling that risk can be expressed as a function of a product of independent variables lacks phenomenological support. An example is provided that shows that this assumption is generally invalid. Conclusion: In view of these problems, we recommend caution in the use of HVD modeling as a general approach to estimating low-dose risks from human exposures to toxic chemicals.

### Proposed Modes of Action for Neurotoxicity Induced by Various Chlorinated Solvents


Several health assessments of chlorinated solvents, including trichloroethylene (TCE), perchloroethylene (PERC), and dichloromethane (DCM), are underway by the U.S. EPA’s Integrated Risk Information System (IRIS) Program. These solvents have been shown to produce similar central nervous system (CNS) effects in animals and humans. The observed neurotoxicological effects for this chemical class are general CNS effects (e.g., locomotor activity changes and anxiety-like effects), visual effects, neurotoxicity, cognitive deficits, sleep cycle disturbances, imbalance, and decrements in nerve function. Since neurotoxicological effects are consistent among the three solvents, we hypothesized that mechanisms producing these neurotoxicological effects may be similar. Available neuropathological and mechanistic studies were evaluated in order to determine which molecular systems may be involved in production of the neurotoxicological outcomes. The mechanistic studies indicate that the chlorinated solvents have several molecular targets which, in turn, explain the observed neurobehavioral effects. PERC and TCE have been demonstrated to interact directly with several different classes of neuronal receptors by inhibiting excitatory receptors/channels and potentiating inhibitory receptors/channel function. Most of the mechanistic studies evaluated effects following acute exposure durations. Given the mechanistic information for TCE, DCM, and PERC, we provide hypotheses for primary targets (e.g. ion channel targets) that appear to be influential in producing the resultant neurological effect. As a result there is uncertainty in extrapolating the acute exposure mechanistic data to chronic neurotoxicological effects following exposures to chlorinated solvents since the molecular targets may be affected differently following a chronic exposure. This analysis of data from multiple chlorinated solvents underscores the need for more research to develop a model of low level chronic human exposures to this class of compounds. These authors’ views do not necessarily reflect the views or policies of the U.S. EPA.

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### Models Used to Support Exposure and Risk Analyses by the U.S. Environmental Protection Agency


In this presentation, we provide an overview of 35 models currently supported and used by the U.S. EPA to assess exposures to human or ecological receptors. An understanding of these models is important because they are often used in addition to or in lieu of monitoring data to estimate environmental concentrations and exposures for use in risk assessments or epidemiology studies and to support regulatory standards and voluntary programs. Information on each model was obtained from several sources, including model documentation (e.g., user manuals, staff papers, external peer reviews), interviews with model developers, and running of the model using real or hypothetical data. The models generally represent the first half of the source-to-outcome continuum and include 12 fate/transport models, 5 exposure models, and 8 integrated fate/transport and exposure models. Many of the exposure models also incorporate cancer or non-cancer risk estimates, including margin of exposure (MOE), hazard index, or toxicity equivalency factors. Each model is summarized with respect to its intended purpose and potential applications, level of analysis and routes of exposure, key data inputs and exposure/risk outputs, temporal and spatial resolution, treatment of variability, and uncertainty, degree of model evaluation, level of internal and external peer review, and interactions with other models. A discussion is also provided regarding recent and ongoing efforts to develop integrated modeling approaches and to perform lifecycle evaluations and retrospective analyses of existing U.S. EPA models. The information presented here should provide a useful up-to-date resource to exposure and risk modelers and practitioners.
Two separate CPs contained up to 47,000 ppm (4.7%) total Pb and 700,000 ppm (70%) total cadmium (Cd). Simulated ingestion extracted 67 μg Cd, which exceeds the U.S. EPA one-day drinking water advisory dose-equivalent of 60 μg/day for a 15 kg child. Simulated mouthing extracted a total of 3.6 μg Cd, compared to the Agency for Toxic Substances and Disease Registry intermediate duration Minimum Risk Level of 7.5 μg/day. While total Pb contents were high, the amounts extracted from simulated ingestion were well below the threshold set by US Consumer Product Safety Commission for evaluating acute exposures and no Pb was detected via salivary extraction. Major traces can be made by a dozen different manufacturers and metal composition among CP samples varies, which could indicate poor quality control during manufacturing and impact chemical properties of metals such as solubility. Other key uncertainties include use of in vitro simulations, and lack of relevant TK data and acute toxicity criteria. Given the potential for leaching toxic metals from CPs, and lack of data for assessing short-term exposures, more information is needed for assessing risk from CPs.

**1933 THE UTILITY OF STUDIES SINCE THE NRC 2001 REPORT ON ARSENIC TO ESTIMATE LUNG AND BLADDER CANCER RISK AT LOW CONCENTRATIONS OF ARSENIC IN DRINKING WATER.**


The National Research Council (NRC) report, Arsenic in Drinking Water ~ 2001 Update, concluded that the human data from southwestern Taiwan (Chen et al. 1992; Chen et al. 1988; Wu et al. 1989) remained the most appropriate data to determine lifetime cancer risk estimates. Since 2001, several studies have examined lung and bladder cancers in persons consuming low concentrations of arsenic in drinking water (≤100 μg/L). We evaluated and compared this recent literature on internal cancers (lung and bladder) induced by arsenic to the NRC (2001) report conclusions with respect to cancer risk at low concentrations of arsenic in drinking water. PubMed was searched for epidemiologic studies, commentary, and reviews pertinent to the NRC lung and bladder cancer risk estimates from low-dose arsenic exposure. Articles published between 2001 and April 2009 were included. Twelve epidemiologic studies on the lung and/or bladder cancer risk and two meta-analyzes of epiphenomena were published. Major uncertainties were determined to be potentially useful data for the analysis. Four commentaries on the NRC's report were included in the review. The ability of the new epidemiologic studies to impact the risks estimated by NRC (2001), including power and sample size, were evaluated. We concluded that these epidemiologic studies lacked either the statistical power or the information necessary to evaluate the bladder and lung cancer risk estimated by the NRC. The Taiwanese studies still remain the best data on which to make estimates of lifetime cancer risk due to exposure to arsenic. [The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S.EPA].

**1935 POPULATION RISK FROM ARSENIC EXPOSURE IN COMMUNITIES LIVING NEAR COAL COMBUSTION WASTE FACILITIES.**

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In 2007, the United States Environmental Protection Agency (EPA) published the results of a probabilistic risk assessment examining risks associated with the leaching of coal combustion waste (CCW) constituents from different types of waste management units (WMUs). The risk assessment presented the 90th and 50th percentile risks associated with drinking water and fish consumption for the population within one mile and downstream of a WMU. Arsenic, a constituent of concern in CCW waste, presents a risk scenario for cancer. Using median and low dose risk scenarios (risks ranged from 0 to 6 x 10-4 [50th percentile] and 2 x 10-2 [90th percentile]), we provide perspective on how these risks relate to a hypothetical number of excess cancer cases across the US. We used EPA's lifetime risk estimates to calculate the expected number of excess cancer cases above background in the population near CCW facilities, which could reasonably be exposed to CCW-derived arsenic via drinking water. We used information about plant location, expected groundwater flow, and number of dwellings downstream of the facility to identify the number of potentially exposed individuals. From EPA's list of 245 WMUs, 85 WMUs were determined to have the potential for downgradient receptors, and 69 of these had visible downgradient dwellings. Using this potentially exposed population and EPA's risk results, we estimated that in the US, there would be one excess cancer case at the 50th percentile, and 35 excess cancer cases at the 90th percentile risk level. Assuming a typical 70-year lifetime, even at the 90th percentile this would equate to less than one excess case annually, on average. We also conducted a refined analysis where we considered dependence on a municipal water supply. Our evaluation, examining both individual and population risks from arsenic in CCW, could help inform risk management decisions regarding the safe storage of CCW.

**1936 IDENTIFYING PREDICTORS FOR BIOAVAILABILITY OF ARSENIC IN SOIL AT MINING SITES.**


Assessing risks due to arsenic (As) in soil uses toxicity criteria based on exposures to As in drinking water, where bioavailability of soluble salts of arsenic is nearly 100%. Bioavailability of As in soil is usually much lower leading to an overestimation of risk. Site-specific relative bioavailability (RBA), the ratio of uptake of soil-bound As to As dissolved in water, can be determined with expensive in vitro feeding studies. Bioaccessibility is the in vitro counterpart of RBA, but it is not always predictive for the in vivo measurement. This research will identify geochemical and mineralogical predictors for bioavailability of arsenic in soil at mining sites. In vitro simulations of biological digestion and leaching will be conducted on soils from sites in Colorado and Nevada to determine the degree of bioavailability and bioaccessibility of As. The wide range of values for bioaccessibility and metal content are adequate to identify gradients and correlations. Further analysis of soil by differential XRD, µ-XRD, µ-X-ray fluorescence, and µ-X-ray absorption will provide chemical and spatial information about the minor mineralogical phases and bioaccessibility of As.

**1937 GENOMIC CHANGES IN HUMAN PRIMARY UROEPITHELIAL CELLS FOLLOWING EXPOSURES TO ARSENITE AND ITS METABOLITES.**

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The Maximum Contaminant Limi (MCL) for inorganic arsenic has recently been decreased from 50 to 10 micrograms per liter. Exposures to concentrations of inorganic arsenic in drinking water on the order of several hundred micrograms per liter and above have been associated with increased incidence of bladder cancer in a number of human populations. This relatively narrow margin of exposure may to some extent be mitigated by a nonlinear dose-response relationship for the carcinogenicity of inorganic arsenic. In this study, human primary uroepithelial cells from 5 subjects were treated in culture for 24 hours with mixtures of arsenite and its metabolites, monomethylarsonic acid and dimethylarsinic acid, at relative proportions (1:1:4) typically observed in the urine of individuals exposed to inorganic arsenic in drinking water. The genomic alterations in these cells were evaluated following exposures to concentrations spanning more than two orders of magnitude in order to elucidate the dose-response for the effects of arsenic treatment on cell signaling pathways potentially associated with carcinogenesis, as well as to identify candidate biomarkers of arsenic exposure and response. There were no significantly (p<0.05) altered genes for treatments at 0.3/0.3/3.1:2 micromolar and below. Eleven genes were significantly up-regulated following the 1:1:4 micromolar treatment. These genes are consistent with responses to oxidative stress (heme oxygenase, glutathione-cystine ligase, and aldo-keto reductase). At the highest concentration treatment (3:3:12 micromolar), 116 genes were altered including the 11 genes responding at the lower concentration. Principal component analysis demonstrated that the effect of variation across individuals was much greater than the changes in expression elicited by arsenic treatment.

**1938 RELATIONSHIP OF MINERALOGY TO BIOACCESSIBILITY OF ARSENIC.**


The wide range of values for bioaccessibility and metal content are adequate to identify gradients and correlations. Further analysis of soil by differential XRD, µ-XRD, µ-X-ray fluorescence, and µ-X-ray absorption will provide chemical and spatial information about the minor mineralogical phases and bioaccessibility of As.

**1939 IDENTIFYING PREDICTORS FOR BIOAVAILABILITY OF ARSENIC IN SOIL AT MINING SITES.**


Assessing risks due to arsenic (As) in soil uses toxicity criteria based on exposures to As in drinking water, where bioavailability of soluble salts of arsenic is nearly 100%. Bioavailability of As in soil is usually much lower leading to an overestimation of risk. Site-specific relative bioavailability (RBA), the ratio of uptake of soil-bound As to As dissolved in water, can be determined with expensive in vitro feeding studies. Bioaccessibility is the in vitro counterpart of RBA, but it is not always predictive for the in vivo measurement. This research will identify geochemical and mineralogical predictors for bioavailability of arsenic in soil at mining sites. In vitro simulations of biological digestion and leaching will be conducted on soils from sites in Colorado and Nevada to determine the degree of bioavailability and bioaccessibility of As. The wide range of values for bioaccessibility and metal content are adequate to identify gradients and correlations. Further analysis of soil by differential XRD, µ-XRD, µ-X-ray fluorescence, and µ-X-ray absorption will provide chemical and spatial information about the minor mineralogical phases and bioaccessibility of As. The wide range of values for bioaccessibility and metal content are adequate to identify gradients and correlations. Further analysis of soil by differential XRD, µ-XRD, µ-X-ray fluorescence, and µ-X-ray absorption will provide chemical and spatial information about the minor mineralogical phases and bioaccessibility of As.
A recent NTP study observed tumors in the oral cavity and small intestine of rats and mice, respectively, following chronic exposure to hexavalent chromium (Cr(VI)) in drinking water at 20 to 180 mg/L. These data have been used for health risk assessment, through application of a linear model and surface area scaling, to estimate risk at environmental exposures occurring at levels approximately one million times lower than those tested in animals. In the absence of mode of action (MOA) data, it has been assumed, because Cr(VI) is genotoxic, that the tumors observed in animals are due to a mutagenic MOA. However, it is also plausible that Cr(VI) does not cause cancer at environmentally relevant exposures because necessary key events require much higher doses. As such, our goal was to develop a MOA framework to identify key events and data gaps. MOAs are likely tissue- and dose-dependent, and key events may contribute to more than one MOA. Key events identified include: 1) competing kinetic processes of absorption and extracellular reduction, 2) intracellular reduction of Cr(VI) to Cr(III), 3) oxidative stress, 4) sustained inflammation, 5) oxidative DNA damage, adduct formation or Cr inter- or intra-strand crosslinks, and 6) tumor formation. Other elements of the MOA framework include genotoxicity, apoptosis, and disruption of the normal Cr(III) homeostasis. Our Cr(VI)/MOA/human relevance framework indicates missing data in several critical areas. Specifically, information on dose-response in the low dose range and temporal sequencing of key events do not exist for measures of oxidative stress, inflammation and other key events. Further, the tissue doses at which many key events occur are entirely lacking and are important for quantifying interspecies variability. Using our MOA/human relevance framework, critical data gaps have been identified and a research plan constructed to provide mechanistic and dosimetric information necessary for human health risk assessment.

1942 MODE-OF-ACTION PROPOSAL FOR ORAL HEXAVALENT CHROMIUM CARCINOGENESIS

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A recent chronic bioassay conducted by the National Toxicology Program (NTP) found that oral exposure to hexavalent chromium (CrVI) in drinking water caused duodenal adenomas in male mice at 90 mg CrVI/L and in female mice at 60 mg CrVI/L. We present an analysis suggesting that the mode of action for CrVI carcinogenesis in mice likely involves regenerative cell growth secondary to tissue injury. Thus, the proposed key event would be the initial tissue injury, and the mode of action is expected to exhibit a threshold dose-response. Support for this mode of action is based on results from a chronic NTP bioassay showing that minimal epithelial hyperplasia of the duodenum in male and female mice at all doses, with the proliferative response occurring at doses lower than those associated with tumors. Also, a 3 month NTP study showed increased diffuse epithelial hyperplasia in the duodenum of mice, providing temporal concordance between these hyperplastic responses and eventual tumor formation. Multiple lines of evidence from the scientific literature provide evidence that chromosomal damage from drinking exposure of CrVI is unlikely. For example, in the case of the NTP genotoxicity study, in two different studies there was no statistically significant increase in in vivo chromosomal damage in the identical mouse strain that was used in the chronic bioassy. (It should be noted that an increase in chromosomal damage was observed in a study involving male mice from another strain.) There is also evidence supporting our proposal from a recent study that found no increased DNA damage in the duodenum of mice following chronic oral exposure sub-tumorigenic doses of CrVI in drinking water. Thus, we conclude that the preliminary evidence supports a non-linear dose-response model for CrVI, although additional MOAs analyses would be important.
Vanadium is primarily used in the production of rust-resistant, spring, and high-speed tool steels; vanadium pentoxide is used in ceramics. ATSDR recently re-evaluated the toxicity of vanadium and released an updated toxicological profile for public comment in October 2009. Although the general population is primarily exposed to vanadium via the diet, urban populations, especially in the Northeast, are exposed to elevated vanadium oxide levels in the air. Limited occupational exposure data suggest that the respiratory tract is the most sensitive target of toxicity of ingested vanadium. Studies in rats and mice exposed to vanadium pentoxide support these findings. Chronic exposure to vanadium pentoxide resulted in several respiratory tract effects (e.g., alveolar and bronchiolar epithelial hyperplasia, chronic lung inflammation, epiglottis epithelial degeneration of human and dog trachea and larynx). Nickel releases from medical devices in humans are much lower and elevated exposures are transient. These factors combine with the primarily high-dose mode of action of soluble nickel indicate low risk of carcinogenicity from nickel in medical devices.

**1944 HEALTH RISK OF INTERNAL NICKEL EXPOSURE FROM MEDICAL DEVICES**


Nickel is a focus of potential health risks from corrosion of medical devices because of its use in many medical-grade alloys, the prevalence of nickel allergy in the general population, and reported tumors in some studies of pure or high nickel-containing powders or pellets implanted in rodents. This review evaluates the available evidence for assessing the potential health effects of nickel released from medical devices. Nickel is generally of low toxicity for consumer exposures, resulting primarily in dermatitis in sensitive individuals from skin contact (about 8-10% of women and 1-2% of men). Oral exposures may also cause contact dermatitis, although typically only in previously sensitized individuals. Nickel oral dosing studies indicate that most sensitive individuals would react to a 1 mg/day systemic dose (adjusted for oral absorption) whereas only 1% of sensitive individuals would react to an approximately 0.1 mg/day, which is within background exposures from diet and water. Tolerance can develop from repeated oral dosing in previously sensitive people (absorbed dose of 0.2 to 0.6 mg/day). Patch testing before and after implantation of medical devices also indicates patients may become more sensitive or less sensitive to nickel. The incidence of nickel allergy from medical devices appears to be less than the incidence of nickel sensitivity in the general population. Medical devices, in most cases, are likely not releasing sufficient nickel to cause such reactions. Systemic nickel exposure may also be less allergenic than by other routes. Although pure nickel or non-medical device alloys with more than 67-68% nickel may cause tumors when implanted in rodents, administered doses are high (e.g., 250 mg/kg). By contrast, carcinogenicity has not been indicated by nickel-containing medical devices in humans or in dogs. Nickel releases from medical devices in humans are much lower and elevated exposures are transient. These factors combined with the primarily high-dose mode of action of soluble nickel indicate low risk of carcinogenicity from nickel in medical devices.

**1945 ATSDR’S CHRONIC INHALATION MINIMAL RISK LEVEL (MRL) FOR VANADIUM.**

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Vanadium is primarily used in the production of rust-resistant, spring, and high-speed tool steels; vanadium pentoxide is used in ceramics. ATSDR recently re-evaluated the toxicity of vanadium and released an updated toxicological profile for public comment in October 2009. Although the general population is primarily exposed to vanadium via the diet, urban populations, especially in the Northeast, are exposed to elevated vanadium oxide levels in the air. Limited occupational exposure data suggest that the respiratory tract is the most sensitive target of toxicity of inhaled vanadium. Studies in rats and mice exposed to vanadium pentoxide support these findings. Chronic exposure to vanadium pentoxide resulted in several respiratory tract effects (e.g., alveolar and bronchiolar epithelial hyperplasia, chronic lung inflammation, epithelial epithelial degeneration, and goblet cell hyperplasia in nasal epithelia). Nickel is known to cause tumors when exposed to ≥0.28 mg V/m3 6 hr/d, 5 d/wk (NTP 2002). Similar lung and larynx effects were observed in mice exposed to ≥0.56 mg V/m3. Benchmark dose analyses of incidence data for respiratory effects observed in male rats were conducted to identify the point of departure for deriving a chronic inhalation MRL for vanadium. The lowest BMDL.10 values ranged from 0.04 to 0.16 mg V/m3. Human equivalent concentrations (HEC) were estimated by multiplying duration adjusted BMDL.10 values by the regional deposited dose ratio (RDDR) for the respiratory tract area of concern. The HECs were 0.0008, 0.017, 0.005, 0.003, and 0.012 mg V/m3 for alveolar epithelial hyperplasia, bronchiolar epithelial hyperplasia, laryngeal inflammation, epithelial epithelial degeneration, and nasal goblet cell hyperplasia, respectively. The HEC of 0.003 mg V/m3 for degeneration of epithelium epithelium was selected as the point of departure for the MRL. This value was divided by an uncertainty factor of 30 (3 for animal to human extrapolation and 10 for human variability) resulting in a chronic inhalation MRL of 0.0001 mg V/m3.

**1946 EVALUATION OF RECENT INFORMATION ON CARCINOGENICITY OF PERCHLOROETHYLENE (PCE) IN HUMANS.**


EPA’s recent evaluation of PCE states that “Overall, the epidemiologic evidence has associated [PCE] exposure with excess risk for a number of cancers, although a causal association has yet to be definitely established.” A number of epidemiological studies support that outcome and support that possibility. For example, a study of urinary PCE concentrations in male veterans of the Vietnam War boiled water. Tolerance can develop from repeated oral dosing in previously sensitive people (absorbed dose of 0.2 to 0.6 mg/day). Patch testing before and after implantation of medical devices also indicates patients may become more sensitive or less sensitive to nickel. The incidence of nickel allergy from medical devices appears to be less than the incidence of nickel sensitivity in the general population. Medical devices, in most cases, are likely not releasing sufficient nickel to cause such reactions. Systemic nickel exposure may also be less allergenic than by other routes. Although pure nickel or non-medical device alloys with more than 67-68% nickel may cause tumors when implanted in rodents, administered doses are high (e.g., 250 mg/kg). By contrast, carcinogenicity has not been indicated by nickel-containing medical devices in humans or in dogs. Nickel releases from medical devices in humans are much lower and elevated exposures are transient. These factors combined with the primarily high-dose mode of action of soluble nickel indicate low risk of carcinogenicity from nickel in medical devices.

**1947 PEAK AND DECLINE OF CANCER RATES AT OLD AGE.**

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Increased age is regularly linked with heightened cancer risk, but modern research suggests a flattening around age 80. Here, new data is reported on all major cancer sites in elderly men and women. We find age-specific cancer incidence rates, mortality rates, and prevalence proportions often reach a maximum at very old age, and subsequently decline. These results were calculated for a single population comprising 9.5% of the United States over the period 1998-2002. We further report that the age of peak cancer incidence (normally around 80) is consistent over the period 1979-2003. Generally, it appears that centenarians are asymptomatic or unsusceptible to developing new cancers. Data was taken from the Surveillance, Epidemiology, and End Results cancer registries, and age population figures derived from censuses, post-censal estimates, and life tables. The age-specific pattern of cancer rates is commonly used to test theories of carcinogenesis. We examine several biological and non-biological reasons for the apparent turnover in cancer rates, including heterogeneous susceptibility to cancer, general senescence, and error in census population figures. We model rising and falling cancer rates with a beta curve obtained by appending an empirical, linearly decreasing factor to the well known Armitage-Doll multistage model. Old age survival data may reveal important details of the relationship between aging and cancer. Additionally, because this relationship may be causal, we suggest that some medical, diet, and lifestyle interventions restricting carcinogenesis ought to be examined for possible effects on longevity.

**1948 STUDY ON THE INHIBITION OF PROTOPORPHYRIN OXIDASE (PPO) FROM RATS, MICE, RABBITS, AND HUMANS.**

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Protoporphyrinogen oxidase (PPO) catalyzes the last common step in the biosynthesis of heme and chlorophyll, a key pathway for both plants and mammals. Activities of liver mitochondrial preparations were based on the spectrophotometric detection of protoporphyrin formation over time. PPO activities were linear to the amount of mitochondria homogenate, and varied across species, with human mitochondria fractions showing lower PPO activity per mg of protein, followed by the rat, mouse and rat. The PPO activity using 20 µg mitochondrial protein of...
human was about 1.5-fold lower (60 fluorescence units/min) compared to rabbit (90 fluorescence units/min) and about 1.8-fold lower compared to mouse (110 fluo-
rescence units/min). The highest PPO activity was obtained with rat mitochondr-
al protein (210 fluorescence units/min), i.e. 3.5 fold higher than in humans. For interspecies comparison of PPO inhibitory potency, IC50 values (50% inhibition 
concentration) were determined. The relative inhibitory potency of BAS 800 H in 
mouse liver mitochondria was highest followed by that in rats. Much lower relative 
inhibitions were seen with human (14.1-fold) and rabbit (16.2-fold), when com-
pared to the rat. These data indicate clear species dependencies of the Kisor® me-
diated PPO inhibition with far stronger inhibition effects in rats and mice than in 
rabbits and humans.

The aryl hydrocarbon receptor (AhR) is a soluble, ligand-activated transcription factor that mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related chemicals. AhR activity has been shown to regulate cell cycle progression both in vitro and in vivo, although the mechanisms are unclear. We have previously shown that exposure to TCDD suppresses liver regeneration fol-
lowing 70% partial hepatectomy (PH). During liver regeneration, the signal trans-
ducers and activators of transcription (STAT) family of transcription factors is im-
portant for driving the coordinated expression of genes needed in hepatocyte proliferation. The goal of this study was to determine if alterations in 
STAT signaling contribute to the suppression of liver regeneration observed in 
TCDD-treated mice. Mice were treated with TCDD (20 μg/kg) or vehicle 24 hr 
before PH and euthanized 12-72 hr after surgery. Remnant liver tissue was ho-
mogenized and analyzed by co-immunoprecipitation and western blotting. Levels 
of phosphorylated STAT1 increased 12 and 24 hr after PH in vehicle-treated mice, whereas phosphorylated STAT1 was not detected in TCDD-treated mice at any 
time point tested. Furthermore, STAT1 communoprecipitated with AhR in the 
generating liver of vehicle-treated mice 12 and 24 hr after PH. In contrast, STAT1 was 
not detected in the AhR complex in TCDD-treated mice at any time point 
tested. Based on these results, it is conceivable that the AhR-STAT1 interaction 
promotes STAT1 activation, and that TCDD treatment abolishes this interaction, 
thereby preventing STAT1 phosphorylation, and inhibiting downstream signaling 
pathways during liver regeneration.

We have recently demonstrated that disruption of ECM/Integrin signaling via 
elimination of the ligin linked kinase (ILK) interferes with signals leading to termi-
nation of regeneration. This study investigates the role of ILK in hepatocyte prolif-
eration induced by Phenobarbital (PB). WT (wild type) and ILK−/− mice 
were given PB (0.1% in drinking water) for 10 days. Livers were harvested on 
2, 5, and 10 day during PB administration. In the ILK−/− mice the liver/body weight ratio was more that doubled as compared to 0h at day 2 (2.5 times) and 5 while at day 10 it was thrice. In the WT mice the increase was not dramatic (1.8 times) and seems to have leveled off after day 2. There were slightly increased PCNA positive cells in the ILK−/− animals at day 2 as compared to WT after PB administration. In the WT animals the proliferative response had come back to normal by day 5 and 10 but the ILK−/− mice still had PCNA positive cells at day 5 and 10 suggesting a pro-
longed proliferative response. ILK−/− mice also showed increased expression of key 
genes involved in hepatocyte proliferation at different time points during PB ad-
ministration. In summarize, ECM proteins communicate with the signaling ma-
achinery of dividing cells via ILK to regulate hepatocyte proliferation and termina-
tion of the proliferative response. Lack of ILK in the hepatocytes has prolonged 
proliferative response to a mitogenic stimuli suggesting defect termination of pro-
liferative response.

Nevirapine, a non-nucleoside reverse transcriptase inhibitor, is used in the treat-
ment of AIDS and the prevention of mother-to-child transmission of HIV-1. 
Despite its therapeutic benefits, treatment with nevirapine has been associated with 
a significant incidence of hepatotoxicity and skin rash. The present study examined 
the effects of nevirapine on cell cycle progression. The human hepatoma cell line 
HepG2 cells were incubated with (0 – 100 μM) nevirapine for up to 4 weeks. A sig-
nificant decrease in the number of viable cells was observed with 100 μM nevrap-
ine at exposures ≤ 1 week and with 20 μM nevirapine after 4 weeks of exposure. 
During a 1-week post-nevirapine recovery period, there was only a slight increase 
in the number of viable cells. Neither necrotic nor apoptotic cell death contributed 
to the decrease in viable cells. Nevirapine primarily produced an accumulation of cells in 
the S phase at exposures ≤ 3 weeks and in the G2/M phase of exposures > 3 weeks, 
with a corresponding depletion in the number of cells in the G1/G0 phase. During 
the recovery period, the disruption of the cell cycle persisted. A Comet assay was 
performed in cells treated with nevirapine for 48 h and 1 week. The tail moment 
was significantly increased after 1 week of exposure. These data indicate that nevi-
rapine suppresses cell cycle progression and that the nevirapine-induced DNA damages might play a role in this delay. (Supported by Interagency 
Agreement 224-07-0007 between NCTR/U.S. FDA and NIEHS/NTP?)

TCDD ENHANCES INFLAMMATORY LIVER INJURY IN RESPONSE TO CONCANAVALIN A ADMINISTRATION.

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a persistent environ-
mental pollutant, has been linked to a number of adverse health conditions. 
Inflammation has been recognized as a component of a variety of chronic diseases. 
We tested the hypothesis that TCDD pretreatment increases hepatotoxicity result-
ning from administration of the inflammatory stimulus, concanavalin A (con A). Ten 
week old mice were pretreated with an oral dose of TCDD or vehicle control 
on day zero and then administered either con A or saline via intravenous injection 
on day four. Mice treated with TCDD did not develop liver injury. Con A treat-
ment alone led to mild hepatotoxicity, TCDD-pretreated mice given con A had in-
creased liver injury compared to either treatment alone, as assessed by liver 
histology as well as elevated alanine aminotransferase (ALT) activity in the 
plasma. Mice pretreated with TCDD had significantly increased plasma ALT activity 
compared to vehicle-pretreated controls at the peak of injury (8 hrs following 
con A treatment). ALT activity remained elevated through 24 hrs in TCDD-pre-
treated mice, whereas in vehicle-pretreated mice it had returned toward baseline 
at this time. In addition, mice pretreated with TCDD had increased plasma concen-
trations of the cytokines, interleukin-6 and interferon gamma, following the in-
flammatory stimulus. These results indicate that TCDD pretreatment enhanced 
The inflammatory response to con A, leading to greater hepatotoxicity. The data 
suggest that TCDD exposure might increase sensitivity to inflammatory stimuli 
and the harmful consequences associated with inflammation. (Supported by NIH 
grant ES04911)

PARP INHIBITOR ATTENUATES COCAINE-INDUCED 
HEPATOTOXICITY.

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Cocaine abuse is a public health hazard in the United States associated with occa-
sional hepatic failure and death. The mechanism of cocaine-induced hepatotoxicity 
(CIH) is not clear, although studies in mice have demonstrated that cocaine-in-
duced liver injury may be mediated by nitric oxide and reactive oxygen species.
Recently, we have found that cocaine increases poly (ADP-ribose) polymerase (PARP) activity in liver. Therefore, inhibition of PARP may block CIH. A prelimi-
nary study showed a partial in vitro (3.4-Dihydroxy-4H-1-chroman-6-yl-3-[4-
(piperidinyl)-1(2H)-isoquinolinone (DPQ) in ICR mice failed to attenuate CIH.
During administration of the inflammatory stimulus, concanavalin A (con A).
Ten week old mice were pretreated with an oral dose of TCDD or vehicle control 
on day zero and then administered either con A or saline via intravenous injection 
on day four. Mice treated with TCDD did not develop liver injury. Con A treat-
ment alone led to mild hepatotoxicity, TCDD-pretreated mice given con A had in-
creased liver injury compared to either treatment alone, as assessed by liver 
histology as well as elevated alanine aminotransferase (ALT) activity in the 
plasma. Mice pretreated with TCDD had significantly increased plasma ALT activity 
compared to vehicle-pretreated controls at the peak of injury (8 hrs following 
con A treatment). ALT activity remained elevated through 24 hrs in TCDD-pre-
treated mice, whereas in vehicle-pretreated mice it had returned toward baseline 
at this time. In addition, mice pretreated with TCDD had increased plasma concen-
trations of the cytokines, interleukin-6 and interferon gamma, following the in-
flammatory stimulus. These results indicate that TCDD pretreatment enhanced 
The inflammatory response to con A, leading to greater hepatotoxicity. The data 
suggest that TCDD exposure might increase sensitivity to inflammatory stimuli 
and the harmful consequences associated with inflammation. (Supported by NIH 
grant ES04911)
Thus, inhibition of both PARP and iNOS appear to be necessary to attenuate CIH. In summary, DIQ is effective in preventing CIH and this is the first time this effect has been reported. The mechanism of action of the DIQ attenuation of CIH appears to be alteration of PARP and iNOS activity. (Supported in Part by Department of Defense Grant W911SR-07-C-0084)

**1954 INVOLVEMENT AND ROLE OF OXIDIZED-LDL (OXLDL) AND ITS RECEPTOR CXCL16 IN THE PATHOGENESIS OF INFLAMMATION-INDUCED MONOCROTALINE (MCT) HEPATOTOXICITY IN MICE.**

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Idiosyncratic xenobiotic hepatotoxicity is a serious clinical problem as it accounts for a remarkable percentage of all xenobiotic-induced liver failure cases. Some reports point to oxidative stress represented by accumulation of oxLDL as a common observation in patients with idiosyncratic hepatotoxicity. The aim of this study was to identify the oxidative damage of liver after MCT administration under a modest inflammatory condition induced by LPS (Lipopolysaccharides) exposure and to explore the role of oxLDL receptor, CXCL16, an excessive chemokine that expressed as a cell surface and a soluble form, in mediating this damage. Methods. For in vivo study, twenty male ND4 mice were randomly assigned into four groups: MCT/LPS, MCT, LPS and control. In the MCT/LPS group, the mice were fasted for twelve hours then dosed with MCT (200 mg/kg) p.o. and after 4 hours injected with LPS (6 mg/kg) i.p. then immediately offered food. For in vitro study, HepG2 (hepatocellular carcinoma cells) THP1 (acute monocytic leukemia cells) cocultures were used. Results. In the present study, we found the following in liver cells of MCT/LPS cotreated group oxLDL was significantly increased; CXCL16 was significantly overexpressed mirroring high levels of oxLDL; α3 integrin expression was significantly suppressed; Fibronectin expression was significantly increased; and, CXCL16 blocking antibody decreased fibronectin and restored α3 integrin expressions. To conclude, regulation of CXCL16 and oxLDL expression may be an early event in the onset of inflammation-induced MCT hepatotoxicity suggesting that both play a important role in the development of hepatotoxicity in this model, therefore both proteins may represent potential new targets for diagnosis, prognosis and management of idiosyncratic drug hepatotoxicity.

**1955 D-AMPHETAMINE-INDUCED CYTOTOXICITY AND OXIDATIVE STRESS IN ISOLATED RAT HEPATOCYTES.**

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Amphetamines (AMP) are potent psychostimulants and commonly used drugs of abuse. The objective of the present study was to investigate the cytotoxic effects as well as the oxidative stress induced by D-amphetamine in rat hepatocytes. Hepatocytes were isolated and exposed to different concentrations of amphetamines in a time-course experiment for up to 2 h. AMP exposure induced a significant decrease in cell viability and a significant increase in the leakage of hepatic enzymes in a concentration and time-related manner. AMP exposure resulted in a significant decrease in cellular GSH content as well as a significant enhancement of TBARS accumulation in a concentration and time-related manner. The obtained results suggested that 2h-exposure of hepatocytes to AMP (0.8 μM) was accompanied by maximal responses. A subsequent dose-response experiment was designed to evaluate the role of GSH modulation and oxidative stress in AMP toxicity in hepatocytes at 2 h. LDH release and TBARS generation were used as indicators in this experiment. Pretreatment with the GSH-depleting agents, chlorodinitrobenzene (CDNB), buthionine sulfoxime (BSO), or biochloroethyl)-nitrosurea (BCNU), enhanced the cytotoxicity of AMP. Pretreatment with GSH or sulhydryl compounds attenuated AMP toxicity. Similarly, co-incubation with enzymatic antioxidants, superoxide dismutase (SOD), or catalase (CAT), or iron chelator, desferroxamine (DFO), or the hydroxyl radical scavengers, mannitol (MAN) or dimethylsulfoxide (DMSO), exhibited significant protection against AMP cytotoxicity. Our results suggest that amphetamines have a potential cytotoxic effect in isolated rat hepatocytes. Thiol group donors, antioxidants, free radical scavengers, and iron chelators can play a critical role against amphetamines-induced cellular damage.

**1956 OXIDATIVE STRESS IS A POTENTIAL PLAYER IN THE PATHOGENESIS OF LIVER INJURY INDUCED BY SULINDAC AND LIPOPOLYSACCHARIDE.**

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Among all the nonsteroidal anti-inflammatory drugs, sulindac (SILD) is associated with the greatest incidence of idiosyncratic hepatotoxicity in humans. Previously, an animal model of SILD-induced idiosyncratic hepatotoxicity was developed by co-treating rats with a nonhepatotoxic dose of LPS. In this study, we further explored the mechanism of liver injury induced by SILD/LPS cotreatment by analyzing gene expression in livers of rats before the onset of liver injury. The results suggested that oxidative stress might be a potential mediator. Moreover, protein carbonyls, products of oxidative stress, were elevated in liver mitochondria of SILD/LPS-coated rats. The potential role of oxidative stress was investigated in vitro. SILD sulfide, the toxic metabolite of SILD, enhanced TNF-induced cytotoxicity and capase 3/7 activity in HepG2 cells. It was observed that SILD sulfide increased fluorescent dichlorofluorescein in HepG2 cells, suggesting induction of oxidative stress in vitro. Compared to either hydrogen peroxide or TNF treatment, hydrogen peroxide and TNF cotreatment caused significant cell death. Either tempol, an antioxidant, or a pan-caspase inhibitor decreased HepG2 cell death as well as capase 3/7 activity induced by SILD sulfide and TNF coexpression. These results indicate that SILD/LPS treatment causes oxidative stress in liver of rats and that reactive oxygen species are important in the cytotoxic interaction of SILD and TNF by activating capase 3/7 in vitro. (Supported by NIH grants R01DK061315 and R11GM075865.)

**1957 TRANSITION METAL-CATALYZED OXIDATIVE STRESS IN ISOLATED RAT HEPATOCYTES: TREATMENT WITH B1/B6 VITAMINS AND METAL CHELATING DRUGS.**

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Iron (Fe) and copper (Cu) are essential trace elements and components of cellular metabolism. When these metals are present in excess, they can participate in redox reactions that lead to the generation of reactive oxygen species (ROS), resulting in oxidative stress conditions. It has been widely suggested that oxidative damage results from the reaction of reduced Fe or Cu with hydrogen peroxide to form hydroxyl free radicals via the Fenton reaction which can damage lipids, proteins and nucleic acids, ultimately causing cell death. As Fe and Cu are capable of catalyzing the formation of ROS, nutrients that possess antioxidant properties may provide protection against Fe- and Cu-induced oxidative stress. In the present study, we compared the molecular mechanisms of Fe versus Cu toxicity in isolated rat hepatocytes by determining the cytoprotective specificity of various agents, including B1/B6 vitamins, metal chelating drugs and hydroxyl radical scavengers. Our results confirmed that there were clear differences between Fe- and Cu-induced oxidative stress and that the therapeutic agents tested had multiple mechanisms of protective action. Pyridoxal (B6) offered protection through its Fe catalyzing and antioxidant properties, as well as through its role as a mitochondrial coenzyme. Pyridoxamine (B6) demonstrated Cu catalyzing and ROS scavenging activity, and thiamin (B1) exhibited antioxidant activity against Fe and prevented against Cu-induced mitochondrial toxicity. Furthermore, the Fe chelators desferoxamine and deferoxamine prevented against Cu toxicity, suggesting the role of endogenous Fe in Cu toxicity. Finally, the antidual effects of the protective agents revealed that Fe toxicity was reversible, while Cu toxicity was irreversible. The ability to prevent toxicity through more than one cytoprotective mechanism of action may prove useful as therapy against multi-oxidative stress diseases or disorders, such as those associated with the metabolic syndrome.

**1958 ALPHA-NAPHTHYLISOThiocyanate-INDUCED BILE DUCT EPITHELIAL CELL INJURY IN VITRO IS ASSOCIATED WITH PROCOAGULANT MICROPARTICLE RELEASE.**

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Bile duct epithelial cells (BDECs) express the transmembrane protein tissue factor (TF), a primary activator of the coagulation cascade. BDEC injury is associated with TF-dependent coagulation that contributes to hepatocellular injury.
Disruption of BDEC integrity in alpha-naphthylisothiocyanate (ANTIT)-treated mice could expose TF to coagulation factors in the blood through the release of small TF-containing membrane vesicles called microparticles (MPs). To this end, we tested the hypothesis that ANIT treatment of BDECs induces the release of TF-positive MPs. Analogous to isolated primary murine BDECs, the transformed human BDEC line MMNK-1 expressed TF activity as indicated by a single-stage clotting assay. ANIT concentrations selected for these studies closely mirrored those previously in the bile of rodents exposed to a large dose of ANIT. ANIT treatment of MMNK-1 cells caused a concentration-dependent (0-20 μM) increase in cell viability. In association with cytotoxicity, the level of procoagulant microparticles increased in the culture medium of ANIT-treated MMNK-1 cells. The MP procoagulant activity was determined to be entirely TF-dependent using a single-stage clotting assay and coagulation FVII-deficient plasma. Interestingly, we found that neither the cell permeable antioxidant, ethyl ester glutathione, nor the pan-caspase inhibitor Z-Vad-fmk (10 μM) affected the cell viability or the levels of procoagulant MPs in the culture medium. Similarly, pretreatment with the calpain inhibitor PD150606 did not affect ANIT-induced microparticle generation in MMNK-1 cells. Taken together, the results suggest that ANIT induced BDEC cytotoxicity is caspase-independent and associated with calpain-independent TF-positive MP release. The release of procoagulant MPs from injured BDECs may contribute to TF-dependent coagulation in livers of ANIT-treated mice.

1959 FRUCTOSE ACTIVATES THE POLYOL PATHWAY IN THE DEVELOPMENT OF METABOLIC SYNDROME


Excessive fructose intake can induce features of metabolic syndrome in experimental animals and man. Fructose intake, primarily in the form of added sugar correlates closely with the epidemics of obesity and diabetes. These observations have led to much interest in the role of exogenous sugars (containing fructose) in the current epidemic of metabolic syndrome. However, fructose can also be generated from endogenous sources via the polyl pathway. Aldose reductase, the rate limiting enzyme of this pathway converts glucose into sorbitol which is the metabolized to fructose by sorbitol dehydrogenase (SDH). Here, we demonstrate that postprandial levels of fructose (1 mM) are able to significantly up-regulate aldose reductase expression within 4 hours after exposure in hepatocytes HepG2 cells (2.6-fold increase in control, p<0.01). SDH expression is further up-regulated at 8 hours after exposure (0.7-fold increase in the first 4 hours, p=0.07 and 2.05-fold increase after 8 hours, p<0.01) indicating that AR is creating the pool of sorbitol needed for SDH over-expression. This up-regulation occurs at transcriptional and translational level since increase in both mRNA and protein expression was observed. The mechanism by which fructose up-regulates aldose reductase is mediated by the ability of this sugar to induce intracellular uric acid and reactive oxygen species (ROS). Scavenging ROS with a NADPH oxidase inhibitor (apocynin) or inhibition of uric acid generation (oxypurinol) dramatically diminished the up-regulation of the enzymes of the polyl pathway. This data strongly suggests that the polyl pathway is activated by fructose. Hence, it is possible that metabolic syndrome, who have postprandial hyperglycemia and hyperuricemia, may have substantial endogenous fructose production that may continue to maintain or accelerate features of the metabolic syndrome even if the original underlying mechanism driving it (exogenous fructose ingestion) is curtailed.

1960 IN VITRO USCIC ACID CONCENTRATION/TIME DEPENDENCY TOXICITY EVALUATION

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Introduction: Uric acid (UA), a dibenzofuran lichen product, is used in the United States for weight loss purposes due to its ability to uncouple mitochondrial oxidative phosphorylation. The Food and Drug Administration (FDA) discouraged its intake after severe liver injury and death were associated with its use. Subsequent studies suggested mitochondrial electron transport chain inhibition as UA’s mechanism of action and time and dose dependent. In this report, we used the Stable Isotope-based Dynamic Metabolic Profiling (SIDMAP) technique to investigate this issue. Methods: Plates of primary rat hepatocytes in media containing 13C6-D-glucose tracer were each treated with UA doses of 0, 1, 5 and 10 μM for 2, 6 and 24 hours. Cells and culture media were collected and analyzed for 13C mass isotope distribution using Gas Chromatography/Mass Spectrometry. Results: 13C02 from media bicarbonate and glutamate data suggested that 10 μM UA is a lethal dose associated with flux disruptions in the tricarboxylic acid (TCA) cycle. UA at sub-lethal doses of 1 and 5 μM: a) increased glucose oxidation at all time points, via the citrate to glutamate arm of the TCA cycle as indicated by increases of 13C evolved CO2 and 13C labeled media glutamate, b) decreased the malate shuttle rate and thus, labeling of media glucose via gluconeogenesis and hepatic glucose production after 24 hours. Conclusion: Our data suggest that UA exerts its primary toxic effects by uncoupling succinate’s oxidation to fumarate which is the complex-II coupled reaction in the TCA cycle. UA is highly toxic by disrupting oxidative phosphorylation in quantities as small as 10 μM. This technique offers 13C2 evolution as a promising drug dose-sensitive breath biomarker, particularly in combination with plasma/saliva glucose and glutamate studies, for assessing cellular toxicity of other chemical agents, including drugs, which disrupt the TCA cycle flux.

1961 MODULATION OF THE HEPATIC ANTIOXIDANT ENZYME ACTIVITIES OF MICE BY DICHLOROACETATE AND TRICHLOROACETATE

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Dichloroacetate (DCA) and trichloroacetate (TCA) were previously shown to induce production of different levels of superoxide anion (SA) in the hepatic tissues of B6C3F1 mice in response to doses ranging from non hepatocarcinogenic-hepato- carcinogenic. The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) have been determined in the tissues of those mice and were correlated with SA production. Groups of B6C3F1 mature mice received DCA or TCA post orally at daily doses of 7, 77, 154, and 410 mg/kg/day for 4 and 13 weeks and SOD, CAT and GSH-Px activities were determined in the hepatic tissue homogenates after the animals’ sacrifice. DCA treatment resulted in SOD inhibition by all of the doses after 4 weeks of exposure and also by doses ranging from 7-77 mg/kg/day in the 13 weeks period, but not at 154 and 410 mg/kg/day. TCA activity was determined on the other hand resulted in SOD stimulation by all the tested doses in both periods. While DCA had no effect on CAT and GSH-Px activities in the 4 weeks period and also in response to 7-77 mg/kg/day in the 13 week period, TCA resulted in stimulation of CAT activity and inhibition of GSH-Px in both periods. SOD is responsible for SA dismutation to H2O2 and H2O2 is inactivated by CAT and GSH-Px. Since DCA resulted in SOD inhibition with no effect on CAT and GSH-Px, the results may indicate that SA production plays an important role in DCA-induced hepatotoxicity/hepatocarcinogenicity. On the other hand, SOD stimulation in response to TCA, together with the inhibition of GSH-Px may indicate the role of H2O2 in TCA-induced hepatotoxicity/hepatocarcinogenicity. Also TCA-induced increases in CAT activity may result in partial degradation of TCA-induced H2O2 production and this may contribute to the lower hepatotoxic/hepatocarcinogenic potential of TCA, as compared with DCA.

1962 PREGNANE X RECEPTOR PLAYS A CENTRAL ROLE IN HEPATIC TOXICITY INDUCED BY A SMALL MOLECULE INHIBITOR OF BETA SECRETASE

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Inhibition of beta-secretase-1 (BACE) remains one of the most attractive hypotheses for interdicting progression of Alzheimer’s disease. A mechanistic investigation into hepatic toxicity induced by a small molecule BACE inhibitor, CPM013 was carried out. CPM013 was studied in 4 and 14 day rat toxicology studies. The most prominent effects in these studies were hepatomegaly with histological correlates of increased mitotic figures, vacuolation, hepatocellular hypertrophy, and subcapsular and non-subcapsular necrosis of hepatocytes. Clin path changes included elevations in AST, ALT, and GGT. Hepatic effects in rats were reproduced in mice with the exception of subcapsular necrosis and elevated GGT. Because CPM013 was found to activate the pregnane-x-receptor (PXR) our hypothesis was that the morphologic changes indicative of hepatic effects were PXR-mediated. To study the role of PXR in the pathogenesis of the liver effects, PXR-null and wild-type (C57/BL/6) mice were treated with either vehicle or CPM013 at 150 mg/kg/day for 4 days. Toxicity endpoints in addition to toxicokinetics and toxicogenomics were included. Treatment of WT mice with CPM013 resulted in a 90% increase in liver weights while liver weights did not increase in PXR-null mice. In addition, most of the morphologic changes were absent in livers from PXR-null mice, except for vacuolation, which was noted in both strains although lesser in severity in PXR-null mice. Gene expression analysis indicated that proliferative signals could be explained by the PXR-agonist properties of CPM013. In contrast, PXR-independent gene expression implicated mitochondrial perturbations, which may have accounted for the hepatocellular vacuolation seen in both strains of mice. Thus PXR was found to play a key role in hepatic toxicity induced by CPM013.
1965 CHARACTERIZATION OF AN IMMORTALIZED MURINE KUPFFER CELL LINE FOR USE IN TOXICOLOGICAL STUDIES.

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Kupffer cells play a critical role in both liver physiology and the pathogenesis of various liver diseases. Isolated primary Kupffer cells have a limited lifespan in culture, and due to the relatively low number obtained, limit their study in vitro. Here, an immortalized murine Kupffer cell line was established from transgenic mice that express the thermolabile mutant tsA58 of the Simian virus 40 large T antigen under the control of the H-2kβ promoter. Primary Kupffer cells were obtained using a three-step procedure: liver perfusion, centrifugal elutriation, and sorting for F4/80+ cells. A subpopulation of cells (ImKC) was identified within the small-intermediate size range. Isolated primary Kupffer cells have a limited lifespan in culture, and due to the relatively low number obtained, limit their study in vitro. Here, an immortalized murine Kupffer cell line was established from transgenic mice that express the thermolabile mutant tsA58 of the Simian virus 40 large T antigen under the control of the H-2kβ promoter. Primary Kupffer cells were obtained using a three-step procedure: liver perfusion, centrifugal elutriation, and sorting for F4/80+ cells. A subpopulation of cells (ImKC) was identified within the small-intermediate size range.
1968 A HUMAN HEPG2 LUCIFERASE ASSAY FOR METABOLICALLY ACTIVATED HEPATOTOXINS AND GENOTOXINS.


Hepatic toxicity remains of major concern for drug failure; therefore a thorough examination of chemically induced liver toxicity is essential for a robust safety evaluation. In this manuscript, we describe a high-throughput GADD45β reporter assay for assessing potential liver toxicity. Most importantly this assay utilizes a human cell line and incorporates metabolic activation. Our high-throughput assay relies upon two different reporter genes co-transfected into the HepG2 cells. The gene encoding Renilla luciferase is fused to the CMV promoter and integrated into the mammalian genome to provide a control for cell numbers. The firefly luciferase gene is fused to the GADD45β promoter and used to report an increase in growth arrest and DNA damage. A dual luciferase assay is performed by measuring the firefly and Renilla luciferase activities in the same sample. Results are expressed as the ratio of the two luciferase activities; increases over the base level (control) are interpreted as induction of the GADD45β promoter and evidence of stress responses due to the xenobiotic treatment. This mammalian dual luciferase reporter has been characterized with and without metabolic activation using positive and negative control agents. We evaluated GADD45β based induction of luminescence using 57 model compounds such as ioniaizid, valproic acid, troglitazone, flutamide, MMS, 4-aminobiphenyl, cyclophosphamide, etc. These test compounds included hepatotoxins and DNA damaging agents; many of which are well known reagents employed as controls in hepatotoxicity tests. Our results show a high level of concordance with known hepatotoxicity, some of which require metabolic activation and others are poorly detected by standard in vitro assays. The GADD45 gene is also strongly inducible by DNA damage; thus this assay may provide a means to detect both hepatotoxins and genotoxins. The GADD45 promoter fused dual luciferase assay represents a valuable addition for the armamentarium for the early detection of hepatotoxic compounds.

1969 TOXICOGENOMIC STUDY OF MICROCYSTIN-LR IN WISTAR HAN RATS.

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Exposure to microcystin produces hepatotoxicity and this study examined acute toxicity and the toxicogenomic profile in the liver. Male rats were administered a single intravenous dose of 0, 1, 10, 50, and 100 μg/kg microcystin-LR in sterile PBS. Selected tissues (blood, liver, kidney) were collected from rats per time point after 30 minutes and 1, 3, and 6 hours following dose administration. Treatment with 50 and 100 μg/kg produced significant changes in the clinical and anatomical pathology of the liver including elevated serum enzymes (ALP, ALT, AST, and SDH), hepatic cell degeneration, and necrosis with congestion. Early pathological changes were apparent at 30 minutes after treatment and necrosis began to develop by three hours. Alterations in gene expression were apparent at 10, 50, and 100 μg/kg, with most changes occurring at 3 and 6 hours in the two highest dosage groups. Up regulation of genes associated with liver damage and necrosis occurred primarily in the 50 and 100 μg/kg groups. Activities of protein phosphatase PP-1 and PP2A were decreased after the administration of at least 10 μg/kg. Differential expression of transcripts coding for PP1 and PP2A were up-regulated in the 50 and 100 μg/kg groups at either 3 or 6 hours after exposure. Microcystin exposure not only produces direct liver damage but also causes subtle changes in toxicogenomic parameters associated with toxicity. This work was supported by NIEHS Contract No. N01-ES-55536.

1970 INHALATION OF AMBIENT TRAFFIC RELATED PARTICULATE MATTER DURING POSTNATAL LUNG DEVELOPMENT INDUCES EARLY AND PERSISTENT PULMONARY AND NEUROINFLAMMATION.


Chronic airway disease and decreased lung function in children exposed to ambient air pollution may be due to repeating cycles of injury and repair which alter normal lung maturation. We hypothesized that children may be at higher risk to adverse effects induced from air pollution as compared to adults because lung development is a long term process and lung growth continues for an extensive postnatal period. Children have a higher minute ventilation and activity and tend to spend more time outdoors. In addition, increasing evidence suggests the inhaled pollutants can similarly induce inflammation and oxidative stress in the brain. 4 and 56 day old C57Bl/6J mice were exposed to ambient “Real World” ultrafine and fine particles. Groups of 4 day old mice were exposed to ambient concentrated particles for 4 hours a day for 4 consecutive days and examined at the end of exposure or allowed to recover until 8 weeks of age. Mice were either sacrificed or re-exposed to ambient concentrated particles for 4 days and examined at the end of exposure. Mice exposed starting at 4 days of age induced pulmonary mRNAs encoding p21 and MnSOD. Re-exposure as a 4 day old mouse induced mRNAs encoding proinflammatory cytokines and chemokines. 4 day old mice exposed for two weeks, and recovered for 6 weeks exhibited evidence of brain inflammation, including the presence of reactive microglia in the hippocampus and reactive astrocytes in ventral midbrain. Inhalation of 10 nm particles resulted in significant accumulation of gold nanoparticles in Liver, Kidney, Spleen as well as three sections of the brain (Olf. Bulb, Cerebellum and Cerebral Cortex) in both 4 and 56 day old mice. Developmental exposure to ambient air pollution leads to sustained pulmonary and brain inflammation. Our observations suggest that air pollutants may contribute not only to respiratory disorders but may also predispose to persistent CNS dysfunction. Funded By: EPA PM Center R-827354, U19 AI-067733, P30 ES-01247 and NIEHS P01 ES11617.

1971 CHRONIC LOW-LEVEL ARSENITE EXPOSURE AND ITS EFFECTS ON CARDIOVASCULAR DEVELOPMENT AND DISEASE.

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Epidemiological studies have shown that a high incidence of cardiovascular diseases and hypertension strongly correlate with elevated arsenic levels in drinking water. An estimated 40% of developmental defects are caused by in-utero exposure to environmental agents such as arsenic. Some of these defects can be fatal, but they are also cause for altered gene expression, cell proliferation and differentiation, leading to structural abnormalities and onset of diseases later in adulthood. Our studies show that low level (50 and 100 ppb) arsenite in-utero exposure through drinking water has an effect on proper heart development in our mouse model, which could predispose the onset of cardiovascular diseases later in life. To understand the mechanism of this alteration, in-vitro studies were done with real time PCR on mouse primary heart organ cultures exposed to 10, 50, and 100 ppb arsenite. Initial data show a decreased expression of genes related to epithelial-to-mesenchymal transition, a critical process in heart development. Arsenite treated mouse embryos (E13.5) were sectioned and analyzed, showing abnormal development of aortic and mitral valves, as well as ventricular myocardial thickening. In in-vivo studies, blood pressure measurements are being obtained using a non-invasive tail-cuff system. Additionally, FVB mice treated with 100 ppb arsenite are then compared to controls over a period of one year. Preliminary results show an increase in arsenite treated mice systolic (+31mmHg ± 12), and diastolic (+23/7 mmHg ± 10) blood pressures. Histological sections will be obtained after a year-long treatment has been completed, and will be analyzed to determine potential pathophysiological changes. These results strongly suggest that arsenic exposure in-utero and early in life might play a key role in the developmental basis and onset of diseases like hypertension and other cardiovascular diseases. (NIH ES 04940; ES06694)

1972 GESTATIONAL LEAD EXPOSURE (GLE) PRODUCES LATE-ONSET MALE-SELECTIVE OBESITY, HYPERGLYCEMIA, AND PARA-INFLAMMATION: RISK FACTORS FOR METABOLIC SYNDROME AND NEURODEGENERATION.

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Obesity is a pandemic as 65% of adolescents and adults are overweight and 30% are obese. Epidemiological studies show a positive association between developmental lead exposure and increased body mass index during adulthood. Recently we reported that GLE produced dose-dependent, late-onset male-specific obesity. Our goals were to measure food-fluid intake and characterize aging male and female GLE offspring. C57Bl/6J female mice were exposed to 55 ppm lead throughout gestation and until postnatal day 10 (PN10): equivalent to human gestation period. Male and female offspring were housed separately on PN21 and body weights were taken at 1, 3, 6 and 9-14 months. Food-fluid consumption was measured...
weekly from 9-14 months. At 14 months (sacrifice), fat weights, fasting blood glucose and plasma TNFα levels were measured. Peak [BPb] was 20–25 μg/dl at PN10 and not different from controls by PN30. GLE produced no differences in pup or dam weights, dam food-fluid intake during GLE, or food-fluid consumption in 9-14 month male or female offspring. Body weight of male GLE mice increased from 9-14 months, relative to age-matched controls. At 14 months, relative to controls, GLE males had increased: 1) body weight: ~25%, 2) abdominal, gonadal and subcutaneous fat weights, 3) fasting blood glucose levels, and 4) plasma TNFα levels. At 14 months, female GLE mice had no differences on any of these measures, relative to controls. These results show that GLE-induced obesity in aging males was not due to increased food intake. GLE produced altered body composition, hyperglycemia and systemic low-grade chronic (para-) inflammation in aged males. These findings are consistent with the Developmental Origins of Health and Disease (DOHaD) paradigm and suggest that low-level GLE is a long-term risk factor for obesity-related metabolic syndrome and possibly neurodegenerative diseases: especially in males. Supported by NIH Grants ES012482, EY07551 and EY07024.

1973 INTERSPECIES APPROACH TO THE ASSESSMENT OF HUMAN SUSCEPTIBILITY TO PHthalate-INDUCED ENDOCRINE DISRUPTION.

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A testicular dysgenesis syndrome has been proposed, suggesting that alterations to the in utero and perinatal hormonal environment may explain temporal increases in male reproductive tract abnormalities. In utero exposure of male rats to the plasticizer di-(n-butyl) phthalate (DBP) reveals suppression of fetal testicular steroidogenesis, while mice remain relatively unaffected. To examine this species-specific sensitivity, a rodent host bioassay consisting of human and rodent fetal testicular grafts was developed to examine toxicant-induced effects on testicular development. Human fetal testes (gestational weeks 10-23, n=16) were cut into mm3-sized pieces, implanted into the renal subcapsular space of nude rat hosts, exposed to 250mg/kg DBP for 1, 2 or 3 days, and removed 6 hours after the last dose. Histopathological analysis revealed a significant increase in multinucleated germ cells (MNG; days 2 and 3 p<0.05; two-tailed t-test), and a non-significant increase in the number of nuclei per MNG and diameter of seminiferous cords. RT-PCR analysis of steroidogenic genes revealed no DBP-induced alterations on any day. Using a similar exposure paradigm, fetal mouse or rat testes were implanted into rat hosts and evaluated for the same endpoints. Similar to the intact response, MNG formation was induced in both species following DBP treatment, yet only rat fetal testes grafts exhibited suppression of steroidogenic gene expression. This suggests that the effects of developmental phthalate exposure on seminiferous cords and steroidogenic gene expression are mechanistically distinct, species-specific and intrinsic to the testis.

1974 FETAL STRESS BY Cigarette SMOKE EXPOSURE IN UTERO PREDISPOses ADULT MAle MICE TO HEPATIC FIBROSIS.

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Adult onset of conditions associated with metabolic syndrome including atherosclerosis, obesity and diabetes has been linked to fetal stress. Whether adult onset of non-alcoholic fatty liver disease is also associated with fetal stress is unknown. Exposure to cigarette smoke (CS) during fetal development and early postnatal life is perhaps the most ubiquitous and hazardous of children’s environmental exposures. To determine if prenatal exposure to CS alters hepatic histology and gene expression in mice, 8-week old female CD1 mice were i.p. injected with ovalbumin (OVA) or PBS alone followed by intranasal OVA challenge. Females were then mated and exposed by inhalation to mainstream CS or filtered air (5 d/wk, 4 h/d) from gestational day 4 to parturition and hepatic parameters examined in the adult offspring. Hepatic collagen staining was significantly increased in male offspring prenatally exposed to CS and OVA (OSM) compared to the other exposure groups. This suggests a synergistic effect between CS and OVA exposure on fibrosis. The increased collagen staining in the OSM offspring was primarily peri-venular which is consistent with the stage 2 level of fibrosis noted for each mouse in the OSM group. In addition, collagen CPI1A1 mRNA levels were significantly increased in OSM offspring compared to non-OVA challenged smoked offspring. The collagen protease inhibitor timp1 mRNA levels were reduced in OVA air exposed offspring compared to their smoked counterparts. For all groups, the ratio between timp1 and mmp2 mRNA levels (but not collagen mRNA levels) correlated significantly with collagen staining, suggesting that decreased collagen turnover was primarily responsible for the increased collagen staining. The combination of maternal OVA sensitization and prenatal CS exposure-induced hepatic fibrosis in male offspring suggests that tobacco use by allergic mothers during pregnancy may predispose children to liver disease later in life.

1975 DEVELOPMENTAL ARSENIC EXPOSURE CAUSES OBESITY, HYPERGLYCEMIA, AND LIVER DISEASE IN MALE MICE IN ADULTHOOD.

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There is increasing evidence that early-life exposures to arsenic can cause disease later in life. However, nearly all of these studies evaluated arsenic exposures at ppm concentrations when most environmentally relevant concentrations of arsenic are one thousandth of these levels. Results from our laboratory indicate that early life exposures to 50 or 500 ppb sodium arsenite (given in maternal drinking water 1 week prior to mating until postnatal day 21) induce obesity, hyperglycemia and fatty liver disease in male C57Bl/6J mice in adulthood. On PNDs 2-14, mice were exposed to 0, 50, 500 or 5000 ppb arsenic by gavage. Male offspring in the 500 ppb group had elevated fasted blood glucose levels on PNDs 84-117. The increase in fasted-blood glucose levels corresponded temporally with the increased body weight in this group; however, male offspring in the 50 and 500 pppb treatment groups had elevated blood glucose levels during an intraperitoneal glucose tolerance test administered on PND 119. Impaired glucose tolerance can be an early indicator of insulin resistance, and consistent with this, insulin levels tended to be higher in arsenic-exposed male offspring as compared to controls on PN120 (1.1 ± 0.3, 1.7 ± 0.7, 3.7 ± 1.8 ng/ml, mean ± SEM, N=3-4 for the 0, 50, 500 ppb groups, respectively). Livers from male mice developmentally exposed to 50 or 500 ppb arsenic demonstrated fatty liver disease that included mixed steatosis, enlarged hepatocytes, and areas of focal degeneration on PN120. Livers from females exposed developmentally to arsenic were not fatty but displayed increased number and size of focal inflammatory lesions on PND 120. Given today’s high rates of obesity, type-2 diabetes, and fatty liver disease, it is important to determine whether developmental exposures to arsenic could be contributing to these disease burdens. Supported by NIH RR16463-04 and Bates College.

1976 Prenatal exposure to cigarette smoke alters regulatory T-cells associated with anti-tumor immune responses in a mouse model.

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Prenatal exposure to cigarette smoke (CS) has been identified, through population-based studies, to increase the risk of preterm delivery, sudden infant death syndrome, intrauterine growth restriction, and low birth weight. Expanding epidemiological data show that early life exposure to CS can also increase offspring vulnerability to certain childhood cancers, i.e., malignant brain tumors, various tumors of the central nervous system, and childhood leukemia and lymphoma. However, the underlying mechanisms responsible for the increased susceptibility to developing cancer in children prenatally exposed to CS remains unclear. Thus, a toxicological study was undertaken to investigate the role of regulatory T-lymphocytes (Treg) in this response. The results demonstrated that 5-week-old female B6C3Fl mice exposed in utero to mainstream CS (at a maternal concentration of 15 mg TSP/m3) from gestational day 4 to parturition (4 hr/d) had a 2-fold greater incidence of EL4 lymphoma cell-induced tumors which grew at a faster rate compared to air-exposed, tumor cell-injected controls. In addition, tumor-bearing mice exposed prenatally to CS had increased percentages of CD4+CD25+Foxp3+ Treg cells in the tumor-draining lymph nodes. Furthermore, as the lymphoma cell-induced tumors grew larger over time, percentages of Treg cells within the thymus, spleen, and tumor-draining lymph nodes were all increased. The data suggest that prenatal exposure to CS enhances the development/migration of suppressive Tregs
in the offspring during tumor development, which may act to abrogate the appropriate immune responses necessary for protection of the host against developing tumors. Inst. For Science & Health

1977 BODY WEIGHT AND AUDITORY STARTLE RESPONSE IN RATS PERMANENTLY CHANGED AFTER REPROGRAMMING OF DIET AND EARLY EXPOSURE TO MEHg.


People from Dutch Famine have been found more obese and at higher risk for chronic diseases like obesity, but also neurodegenerative diseases. Obesity is thought to be linked to reprogramming of (neuro)physiological set points during early development of the nervous system. In the present study it is hypothesized that maternal diet determines bodyweight (BW) and (neuro)development in the offspring and, with that, the vulnerability for environmental exposure to toxic substances. Three groups of female Wistar rats (n=24) were kept on a semi-synthetic control diet (n=24), a high-caloric diet (15% butter oil, n=48) or a caloric restricted (deficient) diet (75% of control diet, n=38) during 6-weeks prematuring, mating (1 week) and gestation (3 weeks) period. Pups were cross-fostered immediately after birth to obtain 5 diet groups, i.e. (gestation/LACTATION): cont/CONT; high/HIGH; def/DEF; def/HIGH and high/DEF. The reprogrammed def/HIGH diet group mimics the situation of the Dutch Famine. A selection of male (n=100) and female (n=100) pups was additionally dosed sc. with saline (control) or MeHg (3 mg/kg BW) from PN2 to PN21. In time, BW was measured and an auditory startle response (ASR) was performed. The ASR measures anxiety over-time; disability to habituate in the ASR test is considered indicative of neurodegeneration. Results: 1) BWs of the offspring differed significantly between the diet groups; effects were slightly exacerbated by exposure to MeHg and maintained during adulthood, especially for the def/HIGH group. 2) Dietary effects on ASR appeared causally related to BW changes. Additional effects of MeHg, i.e. a disability to habituate to the ASR, were also seen especially in the female def/HIGH diet group suggesting similar neural deficits as found in the Dutch Famine casualties. The findings support the hypothesis that maternal diet may increase the vulnerability of the developing offspring to (subtle) environmental exposure like MeHg, ultimately resulting in neurodegenerative diseases.

1978 PRACTICAL ADVANCES IN THE CONDUCT OF IMMUNOTOXICITY TESTING.


Assays for the evaluation of immunotoxicity continue to see increased refinement and application in hazard and risk assessment. The U.S. EPA currently requires the assessment of humoral immune function through the use of the Sheep Red Blood Cell (SRBC) assay and, in certain cases, assessment of innate immunity through the examination of Natural Killer (NK) cell activity. The gold-standard assays for these endpoints are the SRBC plaque forming cell (PFC) assay and the NK chromium-release assay. As these assays have several practical limitations, we explored the application of more modern and flexible approaches for assessment of these endpoints. For the SRBC assay, a commercial ELISA approach was evaluated alongside the PFC approach using a dose response assessment with cyclophosphamide (CYP). The kinetics of the antibody response for ELISA measurements matched previous experience with a peak response observed on day 5. Evaluation of the antibody response to CYP revealed similar sensitivity with the PFC yielding 84% and 99% suppression and the ELISA displaying 75% and 99% suppression at doses of 5 and 20 mg/kg CYP, respectively. For the NK assay, a flow cytometry approach anchored to the procedure of the chromium-release assay was optimized and evaluated. Differences included fluorescent labeling of YAC target cells, and monitoring cytotoxicity directly through propidium iodide labeling and cytometry. The evaluation revealed linear increases in cytotoxicity with increasing effector to target cell ratios (from 5 to 40% cytotoxicity) and significant inhibition with the positive control anti asialo-GM1 (greater than 50% inhibition); responses that are similar to that reported for the chromium release assay. In conclusion, application of an ELISA-based SRBC assay showed similar sensitivity to the traditional PFC approach while offering greater flexibility and standardization in assay conduct. Advantages of the flow cytometry approach to the NK-cell assay include higher signal, lower spontaneous release of signal, and avoidance of isotope waste and expense.

1979 ANALYTICAL VALIDATION AND REAGENT COMPARISON FOR PERIPHERAL BLOOD IMMUNOPHENOTYPING IN BEAGLE DOGS.

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The purpose of this study was to develop and validate peripheral blood immunophenotyping procedures for beagle dogs. The cynomolgus monkey is the most prevalent large animal species used for preclinical testing. Many methods for assessing unique endpoints, including biomarkers, inflammatory responses, and immunotoxicity have been developed for cynomolgus monkeys. The beagle is the second most prevalent large animal species used for preclinical testing, but reagents, methods, and scientific literature for evaluating similar endpoints are not always readily available. The procedures described here have been used to evaluate compound-related effects on the immune system of beagles and were especially important when the beagle was the only biologically relevant large animal model available. The precision, stability, and linearity of methods developed to measure total T cells (CD3+), T helper cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), and B cells (CD3+CD21+) in peripheral blood by flow cytometry were assessed. The assay was linear for all antibody concentrations tested (5, 10, and 20 µL of antibody cocktail) and were directly proportional to the absolute volume of the sample (100 µL blood). Twenty animals were compared to assess interaninal precision and five replicate tests/sample were tested for intrasample precision. The assay was precise with interaninal standard deviations values of ≤ 0.5 E3/L or 7% (for absolute and relative values, respectively) and intrasample precision coefficient of variation values ≤ 5%. Results of stability testing showed that whole blood could be held refrigerated up to 48 hours prior to staining with antibody and yield quality immunophenotyping data. To facilitate dissemination of knowledge, historical control reference ranges for beagle peripheral blood immunophenotyping has been included and reagents for two vendors were compared. In conclusion, immunophenotyping methods have been validated and precisely measure T and B cell lymphocyte populations in beagle peripheral blood.

1980 THE MINI-PIG IN IMMUNOTOXICITY TESTING.


As interest is growing in the use of minipigs as an alternative species to traditional non-rodent species for non-clinical safety and efficacy studies of pharmaceuticals there is also interest in testing the potential immunotoxicity of pharmaceuticals needs to be explored. Therefore, various quantitative and qualitative immunotoxicological endpoints, developed in rodents, were adapted and subsequently implemented in a subacute immunotoxicity study in Göttingen Minipigs®. The animals were treated for 59 consecutive days with clinically used doses of the clinically immunosuppressive compounds Cyclosporin A (CsA, 20 mg/kg/day) or Dexamethasone (DEX, 0.4 mg/kg/day). At several time points, various quantitative and qualitative (immuno)toxicological endpoints were analysed for disclosing possible immunotoxicological effects in Göttingen Minipigs®. From the results obtained, it was clear that the different assays showed variable sensitivity in assessing immunotoxic effects of CsA and DEX. CsA had a clear effect on DTH responses and the KLH-specific IgM and IgG antibody responses. DEX inhibited ex vivo proliferative responses and NK cell activity of PBMCs, and the IgM and IgG antibody responses to KLH which was less pronounced than in CsA-treated animal. At necropsy, macroscopically no deviations were observed other than a reduction in thymus size in DEX-treated animals. The absolute and relative thymus weights of both the CsA- and DEX-treated animals were reduced, which was only significant for the DEX-treated minipigs. Histopathology of the thymus revealed the well known distinct reduction in the cortex : medulla ratio. Splenic weights and histology were not affected. Although some endpoints such as lymphocyte subset analysis still need some attention the results of this study clearly demonstrate that the potential immunotoxicity of pharmaceuticals can be assessed in Göttingen Minipigs®.

1981 MEASURING T CELL RESPONSES IN MONKEYS FOR PRECLINICAL DEVELOPMENT.


Non-human primates (NHPs) are frequently used to evaluate the toxicity of drugs that may modulate the immune system. Although a variety of methods are available to monitor the immune system in NHPs, additional methods are needed to assess the responses of CD8 T cells and CD4 T cells. To monitor T cell responses of
Additional experiments are needed to evaluate the ability of the method to detect the cell cycle. The results of the current study demonstrated that this assay could be used to determine cell proliferation; cell activation still occurred. These findings suggested that furosemide had no effects. Interestingly, although methotrexate markedly inhibited proliferation, cell activation still occurred. Frequencies of CMV-specific T cells were often in the range of 1 out of 1000 total PBMC, which approached frequencies obtained from anti-CD3 stimulation. Variability in the responses between monkeys and in individual monkeys over time is currently being evaluated and validation will begin in the near future. Monitoring antiviral T cell responses should greatly facilitate testing of immunomodulatory compounds in NHPs.

**1982 EVALUATING THE IMMUNOTOXICITY POTENTIAL OF DRUGS BY FLOW CYTOMETRY.**
J. R. Piccotti, J. L. Wardrop, L. Lin and N. D. Collins. Department of Drug Safety, Schering-Plough Research Institute, Summit, NJ.

Clonal expansion of T lymphocytes following exposure to antigen is one of the hallmarks of the adaptive immune response. Evaluating the effects of a drug on T cell proliferation in vitro can provide useful information on its immunotoxicity potential in vivo. In the current study, we developed and tested a T cell proliferation assay using human peripheral blood mononuclear cells (PBMC) labeled with the fluorescent probe, carboxyfluorescein diacetate succinimidyl ester (CFSE). In initial experiments, PBMC were stimulated with an anti-CD3 monoclonal antibody (mAb) alone or in combination with an anti-CD28 mAb for 72 hours. The percentages of proliferating cells and the number of cell divisions were measured by flow cytometry. Stimulation of human PBMC with anti-CD3 mAb induced concentration-related increases in T cell proliferation. Incubation of cells with anti-CD28 mAb further enhanced cell proliferation in a concentration-dependent manner compared to anti-CD3 mAb alone. Up to 5 cell divisions were typically seen following anti-CD/CD28 stimulation. In the second phase of the study, we investigated the effects of known immunosuppressive drugs cyclosporine, methotrexate, rapamycin and dexamethasone on T cell proliferation to test the ability of this assay to detect immunosuppression. A non-immunotoxic drug, furosemide, also was tested as a negative control. All immunosuppressive drugs caused concentration-related decreases in the percentage of T cell proliferation, while furosemide had no effects. Interestingly, although methotrexate markedly inhibited proliferation, cell activation still occurred. These findings suggested that T cells were activated in the presence of methotrexate; however, cells arrested prior to division. This observation was consistent with the known effects of methotrexate on the cell cycle. The results of the current study demonstrated that this assay could be used to assess the immunosuppressive potential and mechanism of drug candidates. Additional experiments are needed to evaluate the ability of the method to detect drug-induced immunoenhancement.

**1983 VALIDATION OF FLOW CYTOMETRY IMMUNOPHENOTYPING FOR PRECLINICAL APPLICATIONS IN NON-HUMAN PRIMATES.**
C. C. Cornwall1, A. R. Macintyre1, N. Pratt1, F. Day1, T. Salewsky1, J. Klaassen1, S. Meyer1 and R. Nagata2. 1SNBL USA, Ltd., Everett, WA and 2Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. Sponsor: E. Ery.

Flow cytometry immunophenotyping involves identification of cells using light-scattering properties and the binding of fluorochrome-tagged monoclonal antibodies (MAbs) specific for cell surface phenotypic markers. The enumeration of subsets of peripheral blood lymphocytes binding these MAbs is important for the identification of drug toxicity within clinical and preclinical evaluations. Published guidelines for human clinical immunophenotyping methods have been described. Unfortunately, accepted industry-standard guidelines for validating similar analytical procedures for preclinical flow cytometry laboratories or utilizing nonhuman primate models are not nearly so well defined. The purpose of this project was to develop quality control measures, method validation, and performance guidelines for the enumeration of lymphocyte cell types in the peripheral blood of cynomolgus monkeys using flow cytometry. Though the guidelines established here are widely applicable, the scope of this poster will focus on total T cells (CD3+), T helper cells (CD3+CD4+), T cytotoxic/suppressor cells (CD3+CD8+), natural killer cells (CD3-CD16+), and B-cells (CD3-CD20+). Consistent interpretation of preclinical flow cytometry data requires regular training of the instrument, the reagents and the analytical procedure. To achieve this, we assessed single vs. multicolor staining, inter- and intra-assay precision, and sample stability for each lymphocyte population. Additionally, QC samples and population summarizations were performed to assure accuracy. According to the FDA’s Good Laboratory Practice (GLP) guidelines, each laboratory is ultimately responsible for developing suitable methods for its own equipment use and analytical procedures; this poster reviews the validation and quality control of all aspects of the operation of preclinical flow cytometry assays in nonhuman primates performed at SNBL USA.

**1984 CD159a: A NEW MARKER FOR IDENTIFYING NK CELLS IN CYCNOLOGUS MONKEY WHOLE BLOOD BY IMMUNOPHENOTYPING.**
A. R. Macintyre1, T. Salewsky1, C. C. Cornwall1, N. Pratt1, F. Day1, J. Klaassen1, S. Meyer1 and R. Nagata2. 1SNBL USA, Ltd., Everett, WA and 2Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. Sponsor: E. Ery.

For immunophenotyping studies, CD16 (FcγRII) has been the marker that has often been used for the detection of Natural Killer cells on cynomolgus monkey whole blood. Problems can arise, however, when attempting to identify the phenotypic changes associated when a potential therapeutic antibody is administered to test animals: the therapeutic antibody can bind to the Fc receptor blocking the binding site of the anti-CD16 antibody, masking the antigen being used for phenotypic analyses. Fortunately, an alternate marker, CD159a (NKGA2) has also been identified as a marker on Natural Killer cells of non-human primates. CD159a (NKGA2) is a member of the Cα– dependent (C-type) lectin family and is a type II transmembrane protein that is always co-expressed with CD39. The CD94/NKG2A complex is also detectable in subpopulations of T cells and cytotoxic lymphocytes. Data will be presented that illustrates the ability to fully validate the use of this marker for the identification of CD59CD159a+ cells in cynomolgus monkey whole blood. Data will be presented that evaluates accuracy and intra- and inter-assay precision with all results meeting the acceptance criteria determined a priori. Data will also be presented for stability on D1, D2 and D3 post draw, storage conditions of ambient temperature and 2-8°C with all testing meeting the predefined acceptance criteria. Results indicate that for testing samples up to three days post draw, storage at 2-8°C is preferred.

**1985 CYCNOLOGUS MONKEY INTRACELLULAR CYTOKINE STAINING AND DETECTION USING FLOW CYTOMETRY.**
F. Day1, C. C. Cornwall1, T. Salewsky1, A. R. Macintyre1, N. Pratt1, J. Klaassen1, S. Meyer1 and R. Nagata2. 1SNBL USA, Ltd., Everett, WA and 2Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. Sponsor: E. Ery.

The ability to detect cytokine production by cells is an important analytical method for researchers since these protein mediators have a significant role in the immunoregulation of leucocytes. Cytokine level alterations can lead to the induction of various disease states. Flow cytometric analysis of intracellular cytokine staining evaluates the ability of lymphocytes, upon stimulation, to produce cytokines. Intracellular staining along with cell surface marker staining allows for the identification and characterization of the individual cell types that produce these cytokines. Stimulation of the cells with strong activators such as PMA and ionomycin is usually necessary to illicit such a response. Other stimulants such as antigens or peptides can also be used to measure antigen-specific re-stimulation in vitro. The objectives of this project were to establish a validated method for cytokine detection using flow cytometry and determine the reliability of using IFN-γ, IL-2 and TNF-α for immunostaining cynomolgus monkey whole blood. This established method can then be used, ex vivo, to measure cytokine levels in the leukocytes of antigen challenged cynomolgus monkeys.

**1986 ANALYTICAL COMPARISON OF BONE MARROW SAMPLE COLLECTION TECHNIQUES AND IMMUNOPHENOTYPING FOR CYCNOLOGUS MONKEYS.**
P. Joshi1, J. E. Arrington1 and R. Haas1. 1Toxicology Study Direction, Covance, Madison, WI and 2Clinical Pathology, Covance, Madison, WI and 3Toxicology Services, Covance, Madison, WI.

The purpose of this study was to compare bone marrow sample collection techniques (aspiration and perfusion) for flow cytometric immunophenotyping and characterization of CD34+ cells from cynomolgus primates. These procedures have
been developed to investigate perfusion bone marrow collection as a serial technical, to evaluate changes in bone marrow cells (in particular CD34+ cells) caused by new pharmaceutical products (either intentionally or unintentionally), and to provide a basis for further investigation of stem cells or other precursor cell populations by flow cytometry. Bone marrow samples were collected from the humerus by perfusion or aspiration and compared with whole blood T cells (total, helper, and cytotoxic), B cells, and natural killer cells to determine the degree of blood contamination in bone marrow samples and to quantify CD34+ cells. Additionally, three aspiration bone marrow sample processing techniques (peripheral blood mononuclear cell isolation, lyse-wash-antibody staining-wash, and antibody staining-lyse-wash) were compared alongside whole blood samples and evaluated by flow cytometric immunophenotyping. The results show that bone marrow samples collected by perfusion had the least amount of whole blood contamination. Both perfusion and aspiration bone marrow samples measured higher levels of CD34+ cells than peripheral blood. All three aspiration bone marrow sample processing techniques were comparable for the cell populations investigated. In conclusion, bone marrow samples collected by perfusion yield a more accurate assessment of resident cell populations; bone marrow samples collected via aspiration or perfusion should be processed via peripheral blood mononuclear cell isolation to eliminate sample particulates and provide cleaner samples for flow cytometry or cell culture applications. CD34+ cells can be evaluated with either perfusion or aspiration bone marrow samples and can be measured in peripheral blood.

1987 EFFECTS OF RBC LYSE PROCEDURE ON ACCURATE MEASUREMENT OF CD16+ NK CELLS IN CYNOMOLGUS MONKEYS.

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Evaluation of lymphocyte subsets in peripheral blood is important in assessing the immunotoxicity potential of drug candidates. We developed a 5-color flow cytometry assay for lymphocyte surface markers CD3, 4, 8, 16 and 20 to identify and enumerate T cell (CD3+CD20-), B cell (CD3-CD20+), helper T cell (CD3+CD4+), cytotoxic T cell (CD3+CD8+) and natural killer (NK) cell (CD3-CD16+) subsets in blood of cynomolgus monkeys. A one-tube method contributed to greater processing efficiency. Intra-/inter-assay precision and variability, accuracy and stability were evaluated to determine the validity of the method. Initial precision experiments revealed lower than expected recovery of CD16+ NK cells in many animals. Subsequent investigation suggested the presence of an interfering substance responsible for the low NK cell numbers. NK cell recovery in samples from monkeys with high values was suppressed when mixed with samples containing low NK cell numbers. This interference was confirmed by demonstrating significantly higher NK cell percentages in samples processed utilizing a lyse-wash protocol to eliminate RBC lysate prior to staining as compared to the standard lyse-staining-wash procedure. The lyse-wash procedure was determined to be a critical step in processing techniques. The contrast between NK cell results obtained by the different processing techniques was significant; CV up to 40%, whereas for other subsets, CV was <10%. Animal-animal (day-to-day) variability experiments for CD3-CD20+ and CD3-CD16+ subsets showed CV up to 40%, whereas for other subsets, CV was <10%. Animal-to-animal variability was <23%, with the exception of CD3-CD20+ and CD3-CD16+ subsets, where up to 41% variability was seen. Accuracy testing showed systematic error of <14%.Optimal stability was demonstrated up to 56 hours for whole blood stored at 2-8°C. Stained samples were stable up to 32 hours at 2-8°C. A significant finding was the contrast between NK cell results obtained by the different processing techniques. The lyse-wash procedure was determined to be a critical step to ensure NK cell recovery. Upon completion of all testing, the 5-color assay was established as acceptable for GLP use.

1988 OPTIMIZATION OF KLH IMMUNIZATION AND VALIDATION OF TDAR IN CYNOMOLGUS MONKEYS USING FK506.

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The T-cell dependent antibody response (TDAR) is the primary humoral immune function test to determine the potential toxic effects of compounds that may have increased probability to alter the immune system; as is required by the regulatory agencies. T-cell dependent primary responses to immunization with keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT) and memory responses to KLH were evaluated in cynomolgus monkeys via measurement of specific IgM and IgG antibodies. A known immunosuppressant, FK506, was used as a positive control to determine whether a dose-dependent effect could be observed in standard toxicology and immunotoxicology endpoints. These endpoints were evaluated in 26 adult cynomolgus monkeys, with the goal of measuring CD16+ NK cells in samples from monkeys with high values was suppressed when mixed with samples containing low NK cell numbers. This interference was confirmed by demonstrating significantly higher NK cell percentages in samples processed utilizing a lyse-wash protocol to eliminate RBC lysate prior to staining as compared to the standard lyse-staining-wash procedure. The contrast between NK cell results obtained by the different processing techniques was significant; CV up to 40%, whereas for other subsets, CV was <10%. Animal-animal (day-to-day) variability experiments for CD3-CD20+ and CD3-CD16+ subsets showed CV up to 40%, whereas for other subsets, CV was <10%. Animal-to-animal variability was <23%, with the exception of CD3-CD20+ and CD3-CD16+ subsets, where up to 41% variability was seen. Accuracy testing showed systematic error of <14%.Optimal stability was demonstrated up to 56 hours for whole blood stored at 2-8°C. Stained samples were stable up to 32 hours at 2-8°C. A significant finding was the contrast between NK cell results obtained by the different processing techniques. The lyse-wash procedure was determined to be a critical step to ensure NK cell recovery. Upon completion of all testing, the 5-color assay was established as acceptable for GLP use.

1989 A 6-WEEK STUDY TO DETERMINE THE ANTIBODY RESPONSE OF FK506-IMMUNOSUPPRESSED CYNOMOLGUS MONKEYS FOLLOWING THE SUBCUTANEOUS ADMINISTRATION OF KEYHOLE LIMPET HEMOCYANIN AND TETANUS TOXOID IN THE PRESENCE OR ABSENCE OF INCOMPLETE FREUND'S ADJUVANT.

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The T-cell dependent antibody response (TDAR) is the primary humoral immune function test to determine the potential toxic effects of compounds that may have increased probability to alter the immune system; as is required by the regulatory agencies. T-cell dependent primary responses to immunization with keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT) and memory responses to KLH were evaluated in cynomolgus monkeys via measurement of specific IgM and IgG antibodies. A known immunosuppressant, FK506, was used as a positive control to determine whether a dose-dependent effect could be observed in standard toxicology and immunotoxicology endpoints. These endpoints were evaluated in 26 adult cynomolgus monkeys, with the course of 41 days of twice daily FK506 doses at 0 mg/kg (KLH with or without IFA), 0.75 mg/kg (KLH with IFA), and 3 mg/kg (KLH with IFA). Cross-site analyses of the TDAR assays using qualitative vs. quantitative methods were compared. TDAR primary and secondary responses were observed in a dose dependent manner in which an almost complete inhibition of the anti-KLH IgG responses were observed in all animals in the high dose group and in most animals in the low dose group. The same trend to a lesser degree was seen with the anti-KLH IgM responses. The comparison of antibodies dosed with or without IFA resulted in minimal differences in measured endpoints. Slight decreases in CD3-CD16+ NK cells count and NK cell cytolytic activity were observed in FK506-dosed animals. Microscopic evaluation verified the FK506 immunosuppressive effects. These results demonstrate that TDAR assays are able to detect dose dependent immunosuppression using the positive control, FK506. This study validates a weight of evidence approach including standard toxicology and immunotoxicology analyses to detect immunotoxicological effects of compounds.

1990 PARTIAL VALIDATION OF A SEMI-QUANTITATIVE ELECTROCHEMILUMINESCENCE (ECL) METHOD FOR THE DETECTION OF ANTI-MAB ANTIBODIES IN FETAL CYNOMOLGUS MONKEY SERUM.

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A semi quantitative ECL method for the detection of anti-mAb antibodies in monkey fetal serum was partially validated. The method was previously validated in normal monkey serum, using the same mouse positive control. The assay is dependent on the bivalent binding of anti-mAb antibodies to both biotin and ruthenium labeled mAb. This bridging assay format eliminates the need for species specific secondary antibodies and detects theoretically all classes of immunoglobulins, as long
as they are functionally multivalent. The ruthenium complex produces light (ECL) on application of an electric potential. Due to matrix limitation, the focus was kept on the key validation parameters, which included: Negative cut-off and cut point factor (CPF) determination, specificity, selectivity and sensitivity. The CPF was set at 1.2263 after testing 10 lots of fetal serum and 1.4669 in normal serum. The specificity tested with 5 lots of fetal serum met the acceptance criteria. The selectivity tested with 5 lots of fetal serum met acceptance criteria when using pooled fetal serum as a reference to calculate recovery but failed when using a pool of normal serum. Thus, for the rest of the validation assays and during sample analysis, positive controls and blanks were prepared in pooled fetal serum. Before the selectivity assessment, 2 sets of blank samples, one in pooled normal serum and one in pooled fetal serum were loaded on each assay. The mean ECL counts obtained for both sets of blanks were comparable (159.29 ECL counts and 154.46 ECL counts for pooled fetal and pooled normal serum respectively). The sensitivity was established as 9.44 ng/mL, based on the positive control, in both normal and fetal serum. The validation data demonstrated that the method is suitable for its purpose and was used to support immunogenicity testing on a reproductive toxicology study.

**1991 COMPARISON OF DELAYED-TYPE HYPERSENSITIVITY MOUSE MODELS UTILIZING KEYHOLE LIMPET HEMOCYANIN (KLH), SHEEP ERYTHROCYTES (SRBC), OR CANDIDA ALBICANS (C. ALBICANS) AS SENSITIZING ANTIGENS.**

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The search for models that provide an accurate assessment of holistic cell-mediated immunity, such as the delayed-type hypersensitivity (DTH) response, is currently receiving much attention, specifically with regard to developmental immunotoxicology. The purpose of these studies was to establish and compare models using KLH, sRBC, and *C. albicans* as sensitizing antigens for their ability to assess a DTH response (utilizing footpad swelling as the endpoint) with minimal confounding factors resulting from antigen-specific antibody production. Models were established by first determining the challenge level, i.e. the amount of antigen that, when injected into the footpad, would produce no greater swelling 24 hours post-injection than the trauma imparted by injection of physiological saline. Next, a sensitization time-course was completed to determine the peak response for each antigen, followed by a dose-response sensitization study. At each sensitization concentration, footpad swelling was measured 24 hours after challenge, and serum from mice in each group was obtained. A dose-responsive increase in footpad swelling was observed in the *C. albicans* model, while antigen-specific antibody levels were not different between challenge only animals (no sensitization) and animals sensitized with any concentration of *C. albicans* (up to 3x10^5 organisms/mouse). In the sRBC DTH, footpad swelling decreased at the high dose (1x10^7 sRBC/mouse), and a corresponding increase in antigen-specific antibody was observed at the high dose. Footpad swelling decreased dose-responsively with increasing KLH sensitization concentration (5-800 μg/mouse), corresponding to a dose-responsive increase in antigen-specific antibody. These results suggest that the *C. albicans* antigen may be a more appropriate model for evaluating cell-mediated immunity, due to the lack of interference from antigen-specific antibody production. Supported in part by NIEHS Contract ES 05454.

**1992 ASSESSMENT OF ACUTE INFLAMMATORY RESPONSES IN CYNOMOLGUS MACAQUE.**


Acute inflammatory responses represent an important consideration in the safety assessment of novel therapeutics. Symptoms include vasodilation, increased vascular permeability, fever, pain, chemotaxis, and tissue damage. A variety of assays were developed to assess these responses in cynomolgus macaque. Histamine, which can indicate vasodilation and increased vascular permeability, and is chemotactic for eosinophils, was detectable very early in the inflammatory response. Eosinophils were detected by flow cytometry using an autofluorescence methodology to separate from other cell populations. The activated complement component C5a can cause acute anaphylactic inflammatory responses and was validated for detection in cynomolgus plasma. A cytokine ‘storm’ is observed following administration of some antibody therapeutics, and a customized panel was validated to identify IFN-α, IL-6, and IL-8 in cynomolgus plasma. These cytokines are known to be involved with acute inflammatory responses, including endothelial activation and increased thrombogenesis. Platelet activation is involved in some aspects of acute inflammation including histamine release, and activated platelets were detected by their increased expression of CD62P and CD41. Early activation of lymphocytes was assessed through expression of the activation markers CD69 and CD25. Finally, macrophages represent an important component of the innate immune system and its mediators such as nitric oxide causes vasodilation and cytotoxicity. Functional assays to detect phagocytic function, including binding to targets, ingestion, and induction of a respiratory burst, were all validated for use in cynomolgus blood. The use of a multi-analytical biomarker approach can provide a better understanding of the inter-relationship of acute inflammatory mediators in disease states for both preclinical and clinical biological drug development programs.

**1993 EVALUATION OF AN IMMUNOTOXICITY SCREENING METHOD IN THE RAT USING TETANUS TOXOID.**

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Immune function testing may comprise of measuring the antibody response to a T-cell dependent antigen (e.g. KLH). However, when testing immunomodulatory biologics (e.g. monoclonal antibodies), there’s the potential to induce anergy or clonal deletion to KLH when co-administered. In such cases a control antigen such as tetanus toxoid (TT) may prove useful when given to the animal prior to treatment with the biologic as it may provide a better understanding of the immune system before and after treatment. This study set out to establish an immunisation protocol that results in a robust antibody response to TT in the rat. TT was administered twice by intramuscular (i.m.) injection, 14 days apart, at 3 different dose levels. Control groups received cycloporin (oral gavage), for up to 35 days starting 7 days prior to TT challenge. Serum samples were collected pre-study, Day 10, 12, 14, 17, 21, 31, 34 and 36, to assess the optimum time point for observing elevated IgM and IgG levels in serum. The presence of anti-TT antibodies in serum was measured using an ELISA method developed and validated at Covance. Lymphocyte sub-set analysis, was performed on whole blood collected pre-study, Days 12, 14, 21 and 36 and spleen cells collected at necropsy. Challenge with TT, at the 15 I.U. dose level on Days 7 and 21, produced a significant level of anti-TT IgM antibodies by Day 14 peaking at Day 17 with a detectable level of anti-TT IgM observed up to Day 36. The anti-TT IgG response at the same dose level developed from Day 17 and increased steadily until a plateau was reached at Days 34 to 36. Challenge with TT at 1.5 and 0.15 I.U., or treatment with cycloporin resulted in little or no specific antibodies to TT. Changes in lymphocyte sub-sets were mostly restricted to animals given cycloporin and limited to a slight decrease in T and B lymphocytes. Two administrations of 15 I.U. of TT (i.m.), 14 days apart, were sufficient to generate a significant specific antibody response in the rat which could be inhibited by co-administration with cycloporin at 20mg/kg.

**1994 VALIDATION OF A SENSITIVE, QUANTITATIVE, AND ISOETYPE-SPECIFIC ELISA FOR DETERMINING T-CELL DEPENDENT ANTIBODY RESPONSE TO KEYHOLE LIMPET HEMOCYANIN IN RAT SERUM.**


Elicitation of T-cell dependent antibody response (TDAR) following immunization with Keyhole limpet hemocyanin (KLH), a protein purified from the mollusk, Megathura crenulata, has been exploited for immunotoxicity testing in non-clinical species. Suppression of antibody formation to KLH is often monitored to determine test article effect on the humoral immune response. As the number of requests for inclusion of TDAR assessment in drug safety evaluation programs has exponentially increased, productivity measures were taken to increase time efficiency, as well as scientific robustness, of the TDAR assessment by validating an ELISA-based KLH TDAR assay utilizing standard curves to derive concentrations of KLH-specific IgM and IgG rather than endpoint titer results. The validation included assessment of assay precision, method robustness, identification of assay acceptance criteria, and specificity. The results were as follows: 1) the assay was specific for evaluating KLH-specific IgM and IgG in rat serum; 2) standard curves were reproducible within the range of 0.008 to 1.0 μg/mL for KLH-specific IgG standards and 0.02 to 5.0 μg/mL for KLH-specific IgM standards; 3) overall accuracy estimates (percent deviation of the QCs) were within ±32% of expected; 4) overall precision estimates for the QCs were less than 15% CV; 5) inter-analyst precision estimates were less than or equal to 10% CV; 6) co-administration of bilirubin or cholesterol, potential serum contaminants, did not interfere with assessment; and 7) hemoglobin did interfere with KLH-specific IgG assessment. Using this ELISA, the observed concentration ranges in KLH-immunized normal rat serum are 5.17
1995 DEVELOPMENT AND VALIDATION OF AN INNOVATIVE MULTIPLEX ASSAY FOR DETERMINING SERUM IMMUNOGLOBULIN (IG) LEVELS IN RATS.


Assessment of serum Ig levels is often utilized to evaluate potential immunotoxicologic effects of a test article or environmental toxicant. Historically, serum Ig has been assessed using a time-consuming, labor-intensive ELISA format. Thus, an alternative, more efficient multiplex serum Ig assay was developed utilizing Luminex's X-map® methodology. This methodology involves bead suspension array technology in which multiple analytes are assessed simultaneously within the same well of a 96-well filter plate. The assay uses polystyrene beads of different fluorescent spectra, each coupled with an analyte-specific antibody. This assay was validated to quantify serum IgG and IgM levels (specificity of available IgA antibodies could not be demonstrated). The validation included assessment of assay specificity, accuracy, precision, method robustness, stability, identification of assay acceptance criteria and normal population reactivity range. The results were as follows: 1) the serum IgG and IgM assays are specific for IgG and IgM without relevant cross-reactivity to each other, IgA, or an unrelated protein; 2) hemoglobin interfered with serum IgG assessment; 3) standard curves were reproducible within the range of 1.14 - 92.59 μg/mL for IgG and 2.20 - 178.00 μg/mL for IgM; 4) overall accuracy estimates (percent deviation of the quantifiable QC s) were within ±15% of expected; 5) total precision estimates for the quantifiable QCs were less than 27%; 6) multiple analysts can perform this assay on multiple instruments; and 7) the completed filter plate may be stored overnight for up to 24 hours at approximately 4°C prior to analysis. Serum Ig concentration ranges for normal rat serum were 2.04 - 13.40 μg/mL for IgG and 0.32 - 8.71 μg/mL for IgM. These validation data demonstrate a specific, accurate, precise and robust method for quantitating IgG and IgM levels in rat serum. Furthermore, this multiplex assay increases throughput of serum Ig analyses by ≥50%, facilitating incorporation into routine toxicologic assessments.

1996 TRANSCRIPTOMIC PROFILE INDICATIVE OF IMMUNOTOXIC EXPOSURE: IN VITRO STUDIES IN PERIPHERAL BLOOD MONONUCLEAR CELLS.


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Investigating the immunotoxic effects of exposure to chemicals usually comprises evaluation of weight and histopathology of lymphoid tissues, various lymphocyte parameters in the circulation and immune function. Immunotoxicity assessment is time consuming in humans or requires a high number of animals, making it expensive. Furthermore, reducing the use of animals in research is an important ethical and political issue. Immunotoxicogenomics represents a novel approach to investigate immunotoxicity ability of overcoming these limitations. The current research, embedded in the EU project NewGeneris, aimed to retrieve a gene expression profile that is indicative of exposure to immunotoxicants. To this end, whole genome gene expression was investigated in human peripheral blood mononuclear cells (PBMC) in response to in vitro exposure to a range of immunotoxic (4-hydroxy-2-nonenal, aflatoxin B1, benzo(a)pyrene, deoxynivalenol, ethanol, malondialdehyde, polychlorinated biphenyl 153, 2,3,7,8-tetrachlorodibenzo-p-dioxin) and non-immunotoxic chemicals (acrylamide, dimethylnitrosamine, 2-amino-3-methyl-3H-imidazo[4,5-F]-quinoxoline, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). Using Agilent oligonucleotide microarrays, whole genome gene expression profiles were generated, which were analysed using Genetica's Expressionist® software. Using Recursive Feature Elimination and Support Vector Machine, an optimal set of 48 genes was identified that distinguishes immunotoxic compounds from the non-immunotoxic compounds. Analysis of the retrieved gene list for enrichment of biological processes showed the gene set to be highly biologically and immunologically relevant. We conclude that we have identified a transcriptomic profile indicative of immunotoxic exposure.

1997 A MODEL TO IMIC THE EFFECT OF TCDD-CONTAMINATED SOIL INGESTION ON IMMUNE FUNCTION.

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A major route of exposure for animals and humans to dioxins, such as TCDD, is ingestion of TCDD-contaminated soils. However, the degree to which TCDD is bioavailable to immune organs or possesses immunosuppressive actions from TCDD-contaminated soils is unknown. Thus, we developed a model using amorphous silica onto which TCDD could be adsorbed, and then delivered the TCDD-adsorbed silica in water to mice or oral gavage. Mice were also sensitized with sheep erythrocytes (SRBC) to stimulate immunoglobulin M (IgM) production. Proof of principle studies demonstrated that although high doses of silica alone enhanced SRBC-stimulated IgM production, TCDD-adsorbed silica potentely suppressed SRBC plus silica-induced IgM production. A further characterization demonstrated that a 50 mg/kg dose of silica did not enhance the SRBC-induced IgM production and was therefore chosen for additional studies. TCDD-adsorbed silica suppressed SRBC-induced IgM production in a dose-responsive manner, in which 5 μg/kg TCDD-adsorbed onto silica suppressed SRBC-induced IgM production to approximately the same magnitude as 6 μg/kg TCDD delivered in corn oil. Moreover, cytochrome P450 1A1 mRNA expression was modestly induced in splenocytes derived from the TCDD-adsorbed silica treated mice. These results suggest that TCDD was bioavailable to immune organs and interacted with the aryl hydrocarbon receptor in the spleen following oral administration of TCDD adsorbed onto amorphous silica. (Supported in part by NIH P42 ES004911).

1998 DIFFERENCES IN THE CIRCADIAN EXPRESSION OF DNA DAMAGE RESPONSIVE GENES CONTRIBUTE TO THE DIFFERENTIAL SUSCEPTIBILITY OF RAT STRAINS TO MAMMARY CARCINOGENESIS.

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To elucidate the role of circadian control on susceptibility to mammary carcinogenesis, we compared circadian mRNA expression profiles of circadian genes (Per1, Per2, Rev-ErbA and Arntl, estrogen receptors (ERα and ERβ) and melatonin receptor 1c (MTNR1A) in mammary glands of the susceptible Fisher 344 (F344) and resistant Copenhagen (COP) rats with or without exposure to N-nitroso-N-methylurea (NMU). Both strains had similar rhythmic expression patterns of Per1, Per2, Rev-ErbA, Arntl, and ERβ mRNA, with weaker rhythmic expression of ERβ and MTNR1A. However, two days after exposure to a single carcinogenic dose of NMU, mammary glands of COP showed enhanced rhythmic expression of Per2 and Rev-ErbA as compared to untreated rats. The enhanced circadian expression of these genes persisted for 30 days. In contrast, NMU disrupted the rhythmic expression of these two genes in mammary gland of F344 rats. NMU disrupted the rhythmic expression of ERα, ERβ, and MTNR1A in both strains of rats. These results indicated that enhanced rhythmic expression of Per2 might contribute to the resistance of COP to NMU-induced mammary carcinogenesis. We next, using qPCR array, demonstrated that after exposure to NMU, mammary glands of COP rats had up-regulated rhythmic expression on 6 DNA damage responsive genes, whereas mammary glands of F344 did not have any up-regulated gene but 8 down-regulated genes, indicating that there are apparent differences in the circadian expression patterns of DNA damage responsive genes between F344 and COP rats responding to genotoxic stress. These results suggest that different circadian gene expression patterns in mammary gland contribute to its susceptibility to NMU-induced mammary carcinogenesis through effects on the DNA damage response. (Supported by NIEHS grants, U19ES011387, P30ES007035, and P50ES005022).
1999 CHEMICAL EXPOSURE AND BREAST CANCER: IDENTIFYING COMPOUNDS OF CONCERN.

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This research was conducted to evaluate in silico methods for identifying and prioritizing compounds of concern that may increase the risk of human breast cancer. Candidates identified would reflect possible mechanistic causality. Hence they could enter a pipeline for subsequent rigorous testing through an experimental methods-based algorithm proposed by the California Breast Cancer and Chemicals Policy Project. Criteria for selection were NHANES biomonitoring data, presence in breast tissue/milk, hormonal activity, compound reactivity, modulation of critical metabolic enzymes or transporters in breast tissue, and interaction with known human breast cancer targets. The primary dataset included 216 compounds associated with mammary tumors in animals (Rudel R, et al. Cancer Supp 2007). A systematic process for filling information gaps was established using Lazar, CAESAR, OECD Toolbox, Toxtree, Pubmed, ToxNet, among others; and MetaDrug from GeneGo. Key molecular targets and activating pathways were identified for each compound using MetaDrug and pertinent published information (Weigelt B, et al. 2001).

The potential for each compound to activate key metabolic enzymes expressed in breast tissue was assessed as suggested by the literature (Williams J and Phillips D. Cancer Res 2006) and QSAR modeling. A majority of compounds tested positive for mutagenicity and carcinogenicity contained structural alerts associated with such activity. Moreover, 11 compounds predicted modulation of human breast cancer-related targets/pathways, and 25 predicted activation of key metabolic enzymes known to play an important role in carcinogen metabolism. This approach provides valuable assistance in the development of a roadmap to guide chemical screening for agents likely to be involved in breast cancer development and progression.

2000 ENVIRONMENTAL ESTROGENS AND BREAST CANCER.

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Breast cancer is a leading cause of cancer-related death among American women. The etiology of majority of breast cancers is multifactorial, of which 95% is environmental and hormonal. Environmental estrogens include polycyclic aromatic hydrocarbons (PAHs) which appear to be carcinogenic by genotoxic mechanisms. Exposure to environmental estrogens leads to adverse health effects such as reproductive dysfunction, developmental disorders, malignancies etc. The synthetic PAH, 3-Methyl-cholanthrene (MC) induces mammary tumors in rodents. MC represents a novel subclass of environmental estrogens that activates both estrogen receptor (ER-α) and aryl hydrocarbon receptor (AhR) signaling pathways. Approximately 60% of all breast cancer patients have hormone-dependent breast cancer, which contains estrogen receptors and requires estrogen for tumor growth. The formation of depurinating estrogen-DNA adducts in hepatic and mammary tissues of rodents exposed to environmental estrogens but none in controls shows that early stage breast cancer cells are dependent on estrogen for proliferation and survival. These studies are highly significant and will help to design endocrine drugs for prevention and therapy of hormonal based breast cancer.

2001 THE FRY GENE IS INVOLVED IN MAMMARY TUMOR PROGRESSION.

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We previously identified the rat ortholog of the Drosophila furry gene, Fry, as a putative Mammary Carcinoma Susceptibility locus, using a backcross between the resistant Copenhagen (Cop) and sensitive Fischer 3/4 strain. To evaluate the suppressor activity of the Fry gene, we transfected the human MDA-MB-231 mammary tumor cell line, which expresses low levels of Fry, with the wild type allele from Cop (termed 231Cfry). Stably transfected clones were then evaluated for differences in cell growth, morphogenesis and tumorigenicty both in vitro and in vivo. In monolayer cultures, the 231Cfry cells grew in cobblestone formation, whereas the original 231 cells retained their typical scattered growth pattern. When cultured in soft agar, the 231Cfry cells showed a 10.5-fold decrease in cloning efficiency, relative to the parental cells. Moreover, the 231Cfry cells formed uniform spherical mammospheres, while the original 231 cells formed dense, amorphous cell colonies. When injected into the intrascapular fat pads of nude mice, 231Cfry cells ectopically expressing Fry showed an 8-fold decrease in tumor volume relative to the parental cells with low Fry expression. Tumors formed by the 231 cell line invaded into the fat pad and skeletal muscle beneath, whereas the tumors formed by the 231Cfry cell line remained subcutaneous. Histopathological analysis revealed that the tumors formed by the 231Cfry cells were encapsulated in a stroma-like envelope. Additionally, analysis of microarray data available for 2,250 human breast tumors (Oncomine 3.0 Cancer Profiling Database) revealed that Fry gene expression was decreased in estrogen receptor negative tumors. Moreover, decreased Fry expression was associated with the most aggressive and poorly differ- entiated histological phenotypes. Together, these data implicate Fry in mammary epithelial cell morphogenesis, tumor suppression and tumor progression. We also observed that Fry and CACNA1D (alpha subunit of the L-Type calcium channel) were positively correlated in 100% of the mammmary tumor samples analyzed, implicating Fry in calcium mediated cell signaling.

2002 CHEMICAL GENETIC APPROACHES TO ELUCIDATE KEY SIGNALING PATHWAYS FOR THE DIFFERENTIATION OF A MOUSE MAMMARY CANCER STEM CELL.

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The mouse mammary tumor virus (MMTV)-Wnt transgenic mouse model generates tumors which express both basal and luminal cell characteristics with individual tumor heterogeneity. Our working hypothesis is that a mammary gland multi- potential proliferative cell is a target of Wnt pathway activation that generates breast cancers within the class of basal-like breast cancers. We have isolated proliferative, bipotent mouse progenitor cells that co-express markers for both the luminal lineage, keratin 8 (K8), and basal lineage, keratin 14 (K14). Preliminary results reveal a dramatic increase in the percentage of the bipotent precursor cells when cultured under low oxygen atmosphere. This change in cell fate is accompanied by an increase in clonal sphere formation efficiency. Cell cultures enriched for these markers generate tumors from the implantation of a single cell into a cleared fat pad. The tumor generated by this cell recapitulates the heterogeneity of the tumor of origin, fulfilling the prerequisite of a cancer stem cell. Currently we are creating a focused mini-library of chemicals to investigate multiple signaling pathways that could potentially influence the differentiation of these cancer stem cells. With automated cell imaging, a high content cell based screening assay of nucleic and immunofluorescent staining of K8/K14 will be used to identify and quantitate three distinct cell types: bipotent progenitor, committed luminal and committed basal cell lineages. In addition, we are currently testing whether the culture conditions that are selective for mouse tumor initiating cells will permit the efficient culture of human breast cancer cells with tumor activating activity. It is our goal to identify compounds that may effectively treat some types of basal-like breast cancers by instructing the stem cancer cells to become benign. Patient-specific cell cultures could become a new tool for testing and evaluating treatment to eliminate or transform the malignant cells into non-tumorigenic cells.

2003 MODULATION OF UP-A, MMPS AND THEIR INHIBITORS BY A NOVEL NUTRIENT MIXTURE IN HUMAN BREAST, CERVIX, AND OVARIAN CANCER CELL LINES.

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Introduction: Proteases play a key role in tumor cell invasion and metastasis by digesting the basement membrane and ECM components. Strong clinical and experimental evidence show that elevated levels of u-PA and MMPs are associated with tumor growth, metastasis and shortened patient survival. Objective: We investigated the effect of a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract on activity of u-PA and MMPs in MCF-7, Hela and ovarian (SKOV3) cancer cell lines.
cell lines (ATCC) were cultured in MEM media with 10% FBS and antibiotics in 24-well tissue culture plates. At near confluence, the cells were treated with NM at 0, 50, 100, 250, 500 and 1000 μg/ml in triplicate at each concentration. U-PA activity was carried out by liberase zymography, MMPs by gelatinase zymography and TIMPs by reverse zymography. Results: U-PA activity was detected in both breast cancer cell lines, showing two bands corresponding to 55 and 33 kD. NM significantly reduced U-PA activity at 250 μg/ml. However, no bands corresponding to U-PA were detected for Hela and SKOV3 cell lines. MDA-MB-231 and MCF-7 showed one band corresponding to MMP-2. Hela showed two bands, an intense band corresponding to MMP-2 and a faint band corresponding to MMP-9. SKOV3 showed only a band corresponding to MMP-2. NM inhibited their expression in all cell lines at 100 μg/ml and blocked expression at 250 μg/ml. Activity of TIMPs was up regulated in all cancer cell lines in a dose-dependent manner. Minimum activity was expressed at 50 and maximum at 1000 μg/ml. Analysis of correlation revealed a positive correlation between U-PA and MMPs and a negative correlation between U-PA and TIMPs. Conclusions: These findings suggest that NM could potentially be developed as a new anticancer agent that inhibits U-PA and MMPs and increases TIMPs.

2004
THE MAMMARY SECRETORY EPITHELIAL CELL SPECIFIC ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPARγ) IN DMBA-MEDIATED BREAST TUMOURIGENESIS.

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Peroxisome proliferator-activated receptor (PPARγ) plays a role in tumourigenesis. Previous studies with PPARγ(+/−) mice suggest PPARγ normally suppresses dimethylbenz(a)anthracene (DMBA)-induced breast, and other tumour, progression; however, the PPARγ-dependent mechanisms and cell types critical to this process remain unknown. We evaluated whether mammary secretory epithelial (MSE) cell-specific PPARγ signaling normally acts to prevent DMBA-mediated breast tumour progression. To do this, we first crossed our previously described PPARγ floxed mice to transgenic mice expressing Cre recombinase under the control of the whey acidic protein (WAP) promoter and generated a colony of conditional MSE cell-specific PPARγ knockout mice (PPARγ-MSE KO mice). Eight week old female PPARγ-MSE KO mice (n=17) and their congenic wildtype (PPARγ-WT) controls (n=7) were mated to induce pregnancy and allowed to lactate for 3 days to ensure WAP promoter activation. Subsequently, all mice were treated by gavage once/week for 6 weeks with 1 mg DMBA and monitored weekly for 25 weeks. Tumour and tissue samples were collected at necropsy, and portions of each were fixed and frozen for future analyses. Preliminary data suggests that total and mammary tumour incidences were modestly (~10%) higher among PPARγ-MSE KO compared to PPARγ-WT controls, although mammary tumour multiplicity (2.1 vs 2.0 tumors/mouse respectively) were not different. Interestingly, PPARγ-MSE KO mice had a decreased median survival (week 17 vs week 23 respectively), a 2-fold decrease in mammary tumour latency, and a 1.6-fold increase in mammary tumour incidence (week 17 vs week 23 respectively), a 2-fold decrease in mammary tumour latency, and a 1.6-fold increase in mammary tumour incidence. More interestingly, PPARγ-MSE KO mice (n=17) and their congenic wildtype (PPARγ-WT) controls (n=7) were not different. This compound has also been shown to be mutagenic and toxic in short-term in vitro tests systems. This study evaluated quercetin for toxicity in the MCF-7 cell line in the presence or absence of benzo[a]pyrene (BaP), a known DNA-reactive carcinogen. In the clonogenic assay, 2.5 × 104 cells were seeded into 25 cm2 flasks in the presence or absence of graded concentrations of quercetin (BaP). In the cytotoxicity block microculture (CBMN) assay, 3 ml aliquots (w2 × 105 cells) was seeded into 30 mm petri dishes containing 20 mm coverslips; cells were treated with graded concentrations of quercetin in BaP for 24 h (Yared et al., 2002). All test agents were evaluated at micromolar (μM) concentrations. Results were obtained from at least three independent experiments and analyzed for significant mean differences using a Student’s t-test. For quantitative real-time transcriptase polymerase chain reaction (RT-PCR), total RNA extraction was performed using the Qiagen RNeasy® kit in combination with Qiagen RNeasy-free DNase kit. Quercetin induced dose-related increases in cytotoxicity in the clonogenic assay and micronucleus formation in CBMN assay. However in the presence of BaP, quercetin exerted significant protective effects demonstrated by enhanced % survival in the clonogenic assay and reductions in micronucleus formation in the CBMN assay. RT-PCR revealed down-regulated levels of expression of P21 and CIP1A1 in cells incubated in the presence of both quercetin and BaP compared to levels of induction noted in cells exposed to BaP alone. This study provides evidence of quercetin-induced genotoxicity/cytotoxicity in vitro. It also points to quercetin-associated protective effects against a prototypical carcinogenic (i.e. BA[P]) that might be important in an epidemiological setting.


2007
SECOND GENERATION CURCUMIN ANALOGS AS NOVEL DRUGS FOR THE TREATMENT OF ER-NEGATIVE BREAST CANCER.

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We have synthesized analogs of curcumin in order to develop potential drugs for the treatment of ER-negative breast cancer. These compounds were examined for their ability to elicit cytotoxicity in ER-negative breast cancer cell lines (MDA-MB-231 (ER-/Her-2-) and SKbr3 (ER-/Her-2+)). This was followed by apoptosis, cell cycle arrest and mechanistic studies, which examined the protein expression of key cell proliferative proteins that are initiated by the epidermal growth factor receptor (EGFR). Six of the derivatives demonstrated increased cytotoxic potency compared to curcumin. Specifically, 2,6-bis((3-methoxy-4-hydroxyphenyl)methylene)-cyclohexane (BMHPC), and other cyclohexane derivatives abbreviated RL90, RL91, RL92, RL53 and RL71 elicited EC50 values between 0.26 and 2.6 μM in MDA-MB-231 and SKbr3 cells. All other compounds examined were less potent than curcumin (EC50 values of 7.57 and 2.35 μM in MDA-MB-231 and SKbr3 cells, respectively). Mechanistic analyses demonstrated that all of the derivatives significantly increased the number of G2/M-phase cells as well as the number of cells undergoing apoptosis compared to control in both cell lines. Furthermore, RL90, RL91 and RL92 significantly decreased the protein expression of the phosphorylated forms of Akt, JNK and mTOR in both
cell lines, while pHer-2 was decreased in SKBr3 cells. Importantly, following a single oral dose (8.5 g/kg) all of the active derivatives were detected in the plasma of female CD1 mice. RI:92 showed the highest plasma levels, which peaked at 590 ng/ml after 30 min and remained over 400 ng/ml after 240 min. These results demonstrate that we have synthesized 6 derivatives that are more potent cytotoxic agents toward ER-negative breast cancer cells than curcumin. Importantly, these drugs are also orally available and thus all of these will undergo further screening for their tumor suppressive properties in our xenograft model of ER-negative breast cancer.

Betulinic acid (BA) is a pentacyclic triterpenoid natural product that inhibits prostate cancer cell and tumor growth through activating prostate-dependent degradation of specificity protein (Sp) transcription factors and Sp-dependent genes. In this study, BA also inhibited growth of ErbB2-overexpressing BT474 and SKBr3 breast cancer cells; and BA decreased expression of Sp proteins. However, in contrast to prostate cancer cells, this response was prostate-independent. BA also downregulated ErbB2 and this was accompanied by decreased ErbB2-dependent kinase activities, and decreased expression of AP2 and YY1, two transcription factors that regulate ErbB2 expression. BA-induced downregulation of Sp1, Sp3 and Sp4 was accompanied by decreased survivin and Sp-dependent gene products. Downregulation of ErbB2 and Sp proteins by BA was inhibited in cells cotreated with cannabinoid receptor 1 and 2 (CB1 and CB2) antagonists, AM251 and AM630 respectively. The mechanism associated with BA mediated response of Sp proteins through CB1 and CB2 was due to decreased microRNA 20a and microRNA 27a, and the subsequent increase in their targeted genes Zbtb4 and Zbtb10 which have been characterized as Sp1 repressors. The mechanism through which BA decreases ErbB2 protein level by CB1 and CB2 is still under study but also indirectly involves transcription factors. BA represents a novel and promising new anticancer drug that targets both ErbB2 and Sp proteins by activating cannabinoid receptors.

Ovarian cancer (OC) is the leading cause of gynecologic cancer death in the USA. Recurrence rates are high after front-line therapy and most eventually die with platinum-resistant disease. Adding hyperthermia to chemotherapeutic agents delivered intraperitoneally (HIPEC) may help to improve outcome although no definitive clinical trials have been performed. We have developed a murine HIPEC model to test a novel combination of cisplatin (CP), sodium arsenite (As) and hyperthermia (43°C). We have established metastatic OC xenograft tumors in nude mice using CP-resistant human OC (A2780/CP70) cells. Tumor-bearing mice were perfused for 1 h with 2 μM CP at 40 μM As at 37° or 43°C. Platinum and arsenic significantly accumulated in tumors during treatment (0 h). Platinum decreased by 24 h after treatment in the presence of arsenic. Systemic platinum accumulation during treatment with CP alone or the combination chemotherapy was in the order: brain<heart<spleen=kidney<liver. By 24 h after treatment, platinum levels in the kidney decreased significantly. Tissue arsenic after treatment was in the order: brain<heart<spleen=kidney<liver. Arsenic levels decreased significantly in all systemic tissues within 24 h after treatment. Western blot analysis of the key DNA repair proteins XPC, XPA and p53 showed tumor-to-tumor and mouse-to-mouse variability. Cells expressing low levels of XPC and XPA are known to be sensitive to DNA damaging agents, suggesting that our assayd tumors expressing low levels of these proteins will be sensitive to cisplatin treatment. Conclusion: the murine HIPEC system may serve as a useful tool to determine the efficacy of novel treatments and the distribution and safety of chemotherapy. The observed tumor heterogeneity in DNA repair protein levels may explain the high rates of recurrence seen in the treatment of women with OC. Therefore inhibiting DNA repair may sensitize tumors to chemotherapy. Supported in part by NIH grants P30ES014443 and R01ES011314; and NSF-EPSCoR grant EPS-0447479.

In the presence of foreign compounds, metabolic homeostasis of the organism is maintained by the liver's ability to detoxify and eliminate xenobiotics. This is accomplished, in part, by the expression of xenobiotic metabolizing enzymes (XMEs), that metabolize and transport xenobiotics and determine whether exposure will result in toxicity. In order to gain a comprehensive understanding of the effects of age on xenobiotic metabolism, we are using DNA microarray analysis to understand the risks posed to older adults by environmental exposures to chemicals. The capacity to appropriately respond to toxicant exposure declines with normal aging and may be exacerbated in individuals with pre-existing conditions. This decline can result in compromised pharmacokinetic and pharmacodynamic responses to environmental exposures encountered in daily activities. Thus our objectives will be to highlight recent studies of altered sensitivities to xenobiotic exposure by aging in a number of tissues and to bring forward substantial new information on what is known about their mechanisms. We will include an overview of molecular pathways that are altered in aging including those involved in xenobiotic metabolism that will include important examples of how aging alters chemical sensitivity in the liver, lung, and brain. This session will be of interest to those studying the impact of modulation of stress pathways on chemical toxicity and risk assessors interested in incorporating data on sensitive subpopulations.

Betulinic acid (BA) is a pentacyclic triterpenoid natural product that inhibits prostate cancer cell and tumor growth through activating prostate-dependent degradation of specificity protein (Sp) transcription factors and Sp-dependent genes. In this study, BA also inhibited growth of ErbB2-overexpressing BT474 and SKBr3 breast cancer cells; and BA decreased expression of Sp proteins. However, in contrast to prostate cancer cells, this response was prostate-independent. BA also downregulated ErbB2 and this was accompanied by decreased ErbB2-dependent kinase activities, and decreased expression of AP2 and YY1, two transcription factors that regulate ErbB2 expression. BA-induced downregulation of Sp1, Sp3 and Sp4 was accompanied by decreased survivin and Sp-dependent gene products. Downregulation of ErbB2 and Sp proteins by BA was inhibited in cells cotreated with cannabinoid receptor 1 and 2 (CB1 and CB2) antagonists, AM251 and AM630 respectively. The mechanism associated with BA mediated response of Sp proteins through CB1 and CB2 was due to decreased microRNA 20a and microRNA 27a, and the subsequent increase in their targeted genes Zbtb4 and Zbtb10 which have been characterized as Sp1 repressors. The mechanism through which BA decreases ErbB2 protein level by CB1 and CB2 is still under study but also indirectly involves transcription factors. BA represents a novel and promising new anticancer drug that targets both ErbB2 and Sp proteins by activating cannabinoid receptors.

Mutations in genes affecting metabolism, stress responses, endocrine signaling, and telomeres can alter the life spans of a number of model organisms. These mutations have revealed evolutionarily conserved pathways for aging that extend life span in response to sensory cues, caloric restriction, or stress. We have mapped a protein interaction network of human homologs of proteins that modify longevity in invertebrate species. This network is derived from a proteome-scale human protein interaction Core Network generated through unbiased high-throughput yeast two-hybrid searches. The longevity network is composed of 175 human homologs of proteins known to function in aging including ARF and ARF1 in yeast, nematode, or fly, and 2,163 additional human proteins that interact with these homologs. To determine whether homologs of human longevity interacting proteins can modulate life span in invertebrates, homologs of 18 human FRAP1 interacting proteins showing significant changes in human aging muscle were tested for effects on nematode life span using RNAi. Of 18 genes tested, 33% extended life span when knocked-down in Caenorhabditis elegans. These observations indicate that a broad class of longevity genes identified in invertebrate models of aging have relevance to human aging. They also indicate that the longevity protein interaction network presented here is enriched for novel conserved longevity proteins.
flammation and xenobiotic metabolism highlighting a range of responses expected from individuals. The Comparative Toxicogenomics Database was used to identify gene-chemical interactions for those genes altered by aging. A number of chemical categories were identified that would be predicted to induce different levels of effects in young and old individuals. Overall, these studies indicate that there are major species differences in the profiles of XMEs affected by aging that could impact chemical toxicity. (This abstract does not necessarily reflect U.S. EPA policy).

AGING RATS ARE PROTECTED FROM CHLORODECON AMPLIFIED PROGRESSION OF CARBON TETRACHLORIDE HEPATOTOXICITY. H. M. Mehendale. Toxiciology, University of Louisiana at Monroe, Monroe, LA.

The prevailing general perception is that aging increases drug- and chemical-induced hepatotoxicity. The mechanism of many age-dependent adverse drug reactions and hepatotoxicity are not known. Among the different models used to study hepatotoxicity, chlordécone (CD) + CCl₄ interaction model is the most potent: toxicity occurs at individually non toxic levels, extensive liver damage leads to hepatic failure and death, and the mechanisms have been studied at length. In 3 months old adult rats, this interactive toxicity results in 67-fold amplification of hepatotoxicity and lethality. However, 14 months old F344 and 24 months old SD rats are completely resistant to the interactive hepatotoxicity and lethality of CD + CCl₄. The toxic effects of this biological interaction in 3 months old adult rats include extensive hepatotoxicity characterized by elevated ALT, AST, OCIDH serum enzyme levels, hepatic necrosis, bankrupt cellular energy and failed tissue repair leading to progression of injury, hepatic failure, and death. In contrast, in aged rats, only transient hepatic damage was observed even though the initiation of bioactivation-mediated liver injury of CCl₄ in CD pre-exposed rats is the same as in the adults. Proteases such as calpain and phospholipases such as secretory phospholipase A₂, (death proteins), known to spread liver injury may be silenced in advanced age, explaining why the initiated liver injury does not progress. The mechanisms underlying this remarkable resiliency in advanced age against the highly amplified toxicity of CCl₄ in Cd treated rats are of significant clinical interest. These findings suggest that progression of liver injury is prevented as a consequence of overexpressed endogenous inhibitors (calpastatin and annexins) of death proteins by stimulated liver regeneration and tissue repair in advanced age.

COMPARATIVE EFFECTS OF THE ORGANOPHOSPHORUS INSECTICIDES CHLORPYRIFOS AND PARATHION IN ADULT AND AGING RATS. C. N. Pope¹, J. Liu² and N. Mirajkar¹, ². ¹Physiological Sciences, Oklahoma State University, Stillwater, OK and ²Pharmaceutical Sciences, Texas Tech University, Amarillo, TX.

We compared dose-related effects of two organophosphorus insecticides (OPs), parathion (PS) and chlorpyrifos (CPF), in adult (3 mo) and aging (18 mo) rats. Radiotransmitters were surgically implanted for radiotelemetry of physiological endpoints (heart rate, body temperature, motor activity). Following baseline measurements, rats were treated with either PS (adult: 0, 9, 13.5 or 18 mg/kg, sc; aging: 0, 3, 4.5 or 6 mg/kg, sc) or CPF (both age groups: 0, 28, 140 or 280 mg/kg, sc). Biochemical (acetylcholinesterase and butyrylcholinesterase), functional (involutionary movements and SLUD signs) and physiological data were then collected for 96 h. There was greater atrial cholinesterase inhibition in aging compared to adult rats treated with the lowest dosage of either OP, but relatively similar inhibition between age groups with higher dosages. PS elicited tremors in aging (at 6, but not 3 or 4.5 mg/kg) and adult (at 18, but not 9 or 13.5 mg/kg) rats. Few signs of cholinergic toxicity were noted in either aging or adult rats following CPF, however. Relatively similar changes in heart rate (prolonged reduction with little recovery) were noted in both age groups with high dosages of either OP. Temperature was reduced in both age groups, but aging rats showed lesser recovery. Motor activity was initially reduced in both age groups by the highest dosage of either OP but aging rats showed hyperactivity 96 h after exposure. We conclude that aging rats are more sensitive than adults to PS toxicity based on biochemical, functional and physiological endpoints. While both age groups showed relatively similar physiological changes early after exposure, age-related differences in temperature and motor activity were noted later. Finally, although both adult and aging rats show marked OP-related differences in cholinergic signs, they exhibit relatively similar OP-related changes in physiological endpoints recorded by telemetry (Supported by Oklahoma State Board of Regents and grant ES009119 from NIEHS.)

AGING RATS. 2013

PULMONARY EFFECTS OF INHALED AIR POLLUTANTS IN ELDERLY MICE: ROLE OF OXIDATIVE STRESS AND INFLAMMATORY CYTOKINES.

One of the most sensitive populations to the adverse health effects of inhaled air pollutants is the elderly. Mechanisms underlying this increased susceptibility are unknown. Aging is associated with deterioration of both innate and adaptive immunity. Most notable changes include age-related deficits in macrophage and neutrophil activity, which are thought to contribute to increased susceptibility of the elderly to respiratory infections. Aging is also associated with reduced activity of antioxidants resulting in oxidative stress. Studies in our laboratory have focused on analyzing inflammatory and antioxidant responses to air pollutants as potential mechanisms underlying increases in the sensitivity of the elderly to inhaled air pollutants including ozone and particulate matter. Following acute exposure of older, but not younger rodents to these pulmonary irritants, we noted significant structural alterations in the lungs, including patchy thickening of the alveolar septa and inflammatory cell localization in alveolar spaces. Significant increases in NOx in bronchoalveolar lavage fluid were also noted in older mice, as well as lung expression of lipocalin 24p3, an oxidative stress marker, with no effects in younger mice. Exposure to air pollutants resulted in a marked upregulation of TNF-alpha in lungs of both younger and older mice; however this was attenuated in older animals. Whereas increases in lung IL-6 were noted in both older and younger mice, IL-8 only increased in older animals. In younger mice, constitutive expression of manganese superoxide dismutase (SOD) decreased after exposure, while in older mice, constitutive SOD was not detectable and pollutant exposure had no effect on expression of this antioxidant. Taken together, these data suggest that age related alterations in antioxidant defense and production of inflammatory mediators may contribute to enhanced susceptibility of the elderly to pulmonary toxicants. Supported by NIH grants ES004738, ES050022, GM034310, CA132624 and AR055073.

AGING RATS. 2013

TRPPING THE SENSOR: THE ROLE OF TRP CHANNEL SIGNALING IN CARDIOPULMONARY TOXICITY. J. M. Morris¹, D. L. Conklin¹, J. Norris¹, A. Caceres¹, L. Lee¹ and R. Willette². ¹Medicine, University of Louisville, Louisville, KY, ²Pharmacology, Yale University, New Haven, CT. 1Pharmacy, University of Connecticut, Storrs, CT, ³Physiology, University of Kentucky, Lexington, KY and 4Heart Failure DPU, GlaxoSmithKline Pharmaceuticals, King of Prussia, PA.

Transient receptor potential (TRP) ion channels comprise a large family of cationic (calcium) conducting channels (TRP A, C, M, V) responsive to environmental and endogenous stimuli. TRP channels are activated by a variety of compounds such as tear gas and reactive aldehydes, as well as endogenously generated unsaturated aldehydes associated with tissue injury and inflammation, including α, β-unsaturated aldehydes like acrolein and 4-hydroxynonanoyl. The TRP receptor channels transduce a variety of chemical signals via unique receptors (C-fibers) into sensory signals, including pain (nociceptive) responses. Increasingly, these channels are being found in other cell types, including airway epithelial and cardiovascular endothelium. The TRPA1 and TRPV1 (vanilloid- or capsaicin-sensitive) channels are implicated in pulmonary inflammation and asthma associated with exposure to noxious stimuli including chlorine, tear gas, isocyanates, tobacco smoke, and aldehydes. TRP channel activation triggers the release of neuropeptides such as substance P and CGRP, which stimulate local inflammatory responses, vasodilatation, and edema. Recent work extends these findings to include complex cardiovascular responses, such as circulatory collapse and hypotension. These responses are triggered by specific TRP agonists, as well as by unsaturated aldehydes, which implicate a role of TRP channels located in cardiovascular cells or sensory afferents in these tissues in these effects. This session will explore several pathophysiological models that implicate various TRP channels in deleterious effects of noxious stimuli in cardiopulmonary toxicity and probe the mechanisms that connect channel activation/inhibition in these responses to exogenous and endogenous stimuli. The relevance of TRP signaling to human health and the potential for therapeutic targeting will be addressed.

TRPA1 MEDIATES THE NOXIOUS EFFECTS OF TEAR GASES AND INDUSTRIAL ISOCYANATES. J. Jordt. Pharmacy, Yale University, New Haven, CT.

TRPA1, a transient receptor ion channel expressed in chemo sensory neurons, was initially characterized as the receptor for the pungent natural product, mustard oil (allyl isothiocyanate). Using Ca²⁺ imaging and electrophysiological techniques, we...
found that TRPA1 is strongly activated by tear gas agents (CS, CN and CR) and industrial isocyanates such as methyl isocyanate, the toxicant released during the industrial accident in Bhopal, India. In mice, genetic ablation or pharmacological inhibition of TRPA1 dramatically reduced isocyanate- and tear gas-induced nociceptive behavior. We conclude that TRPA1 is the main sensory neuronal target of tear gas agents and isocyanates.

2018 TOBACCO SMOKE, TRPA1, AND ENDOTHELIUM DYSFUNCTION: ROLE OF ACROLEIN.
D. J. Conklin, Medicine, University of Louisville, Louisville, KY.

Although tobacco smoke increases the risk of cardiovascular disease in both active and passive smokers, the components in smoke and mechanisms responsible for increased risk are not elucidated as yet. Activation of TRPA1 channels by α,β-unsaturated aldehydes appears responsible for tobacco smoke-induced pulmonary inflammation and airway hyperreactivity, but the role of TRPA1 receptors in cardiovascular disease associated with cigarette smoke exposure is not clear. Recently, we showed that exposure to tobacco smoke or acrolein, a potent α,β-unsaturated aldehyde in tobacco smoke, stimulates endothelial dysfunction in a gluthathione S-transferase F (GSTF)-deficient mouse model indicating that an aldehyde(s) in tobacco smoke is sufficient to induce endothelial injury in this model. We will discuss the role of TRPA1 channels in mediating the cardiovascular effects of tobacco smoke and acrolein exposure on the endothelium.

2019 TRP RECEPTORS AND SENSORY IRRITATION.
J. B. Morris, School of Pharmacy, University of Connecticut, Storrs, CT.

Studies were performed to assess the importance of TRP receptors relative to nasal trigeminal nerve stimulation by irritants. Stimulation of these nerves initiates the sensory irritation response. Exposure to the known TRPV1 agonist capsaicin caused a marked sensory irritation response that was absent in a TRPV1 knockout strain and was ablated by the TRPV1 antagonist 5-iodoresinaferatoxin. In contrast, the sensory irritation responses to acrolein, acetic acid and styrene vapors were identical in TRPV1 knockout versus wild-type mice and were unaltered by 5-iodoresinaferatoxin indicating the TRPV1 receptor does not contribute to trigeminal activation by these common indoor air pollutants. The α,β-unsaturated aldehyde(s) in tobacco smoke is sufficient to induce endothelial injury in this model. The role of endothelial TRPV4 channels in heart failure, hypertension, and barrier function. Recent evidence suggests that endothelial TRPV4 channels are underlie all of the circulatory events. In the endothelium, TRPV4 channels regulate prostanoid generation, eNOS activity and the cytoskeleton to influence paracrine signaling. The role of endothelial TRPV4 channels in heart failure, hypertension, and barrier function. Recent evidence suggests that endothelial TRPV4 channels are.

2020 ROLE OF TRPA1 IN AIRWAY INFLAMMATION AND HYPERREACTIVITY.
A. Caceres, Pharmacology, Yale University, New Haven, CT. Sponsor: D. Conklin.

Asthma is an inflammatory disorder caused by airway exposures to allergens and chemical irritants. We examined the role of the airway irritant sensor TRPA1 in allergic asthma in the murine ovalbumin model. TRPA1-activating stimuli such as cigarette smoke, chlorine, aldehydes and scents are among the most prevalent triggers of asthma. Our data suggest that TRPA1 is a key integrator of interactions between the immune and nervous systems in the airways, driving asthmatic airway inflammation following inhaled allergen challenge.

2021 ROLE OF TRPV1 IN AIRWAY HYPERSENSITIVITY INDUCED BY MUCOSAL INFLAMMATION.
L. Lee, Physiology, University of Kentucky, Lexington, KY. Sponsor: D. Conklin.

Airway hyperresponsivity is a common pathophysiological feature in various airway inflammatory diseases such as asthma and bronchitis. Increasing evidence suggests that activation of the transient receptor potential vanilloid type 1 receptor (TRPV1) plays an important part in the manifestation of various symptoms of airway hyperresponsivity. Recent investigations have revealed several potential contributing factors to the increase in TRPV1 sensitivity in pulmonary sensory neurons during airway inflammatory reaction. A recent experiment has further demonstrated that allergen sensitization-induced airway inflammation markedly enhanced the sensitivity of pulmonary C-fiber afferents and, more importantly, induced TRPV1 expression in myelinated pulmonary afferents that normally do not exhibit capsaicin sensitivity. Therefore, a better understanding of the mechanisms underlying the increase in sensitivity and/or expression of TRPV1 during acute and chronic airway inflammation should generate the necessary information for developing effective therapeutic interventions to alleviate airway hypersensitivity.

2022 THE ENDOTHELIAL TRPV4 CHANNEL: PHARMACOLOGY, TOXICOLOGY, AND THERAPEUTIC TARGET.
R. N. Willette, Heart Failure DPU, GlaxoSmithKline Pharmaceuticals, King of Prussia, PA. Sponsor: D. Conklin.

This lecture will describe the unique properties of pharmacologic agents developed to selectively probe the functions of the TRPV4 channel. The consequences of selectively activating and blocking endothelial TRPV4 channels will be reviewed and the evidence supporting TRPV4 as a therapeutic target will be explored. Systemic activation of TRPV4 causes a complex hemodynamic response culminating in acute right heart failure and cardiovascular collapse. The events leading to this calamity include: elevation in pulmonary pressure secondary to an ARDS-like reaction in the lung, endothelial-dependent vasodilation and redistribution of fluid to the intestinal submucosal space. It appears that activation of endothelial TRPV4 channels underlie all of the circulatory events. In the endothelium, TRPV4 channels regulate prostanoid generation, eNOS activity and the cytoskeleton to influence paracrine signaling. The role of endothelial TRPV4 channels in heart failure, hypertension, atherosclerosis, and angiogenesis will be discussed.

2023 ZEBRAFISH MODELS FOR DEVELOPMENTAL NEUROBEHAVIORAL TOXICOLOGY.
E. D. Levin1 and S. Padilla2. 1Department of Psychiatry, Duke University Medical Center, Durham, NC and 2Integrated Systems Toxicology Division, U.S. EPA, Research Triangle Park, NC.

With the emerging techniques for toxicology in the 21st century, zebrafish can provide a key mechanistic model for developmental neurobehavioral toxicology because they have already become the predominant aquatic model for the study of molecular aspects of development in general and neurodevelopment in particular. Developmental neurobehavioral toxicology reveals how chemical exposures can alter brain function and behavior in a manner that can be transmitted to future generations. The zebrafish model is an ideal system for studying the effects of toxicants on development and behavior because they have already become the predominant aquatic model for the study of molecular aspects of development in general and neurodevelopment in particular. With these considerations in mind, we will discuss the utility of zebrafish as a model for developmental neurobehavioral toxicology.
w ide many contributions: with its genome sequenced one finds many common genes, even syntenic relationships in terms of how its genes are organized in chro mosomes when compared with the human genome; its fertilization and embryonic growth outside of the mother allows one a very early window of investigation not conveniently available in the long term in mammalian models. Its limited growth in size embryonically allows one to use real-time microscopic techniques to study the development of the nervous system throughout and beyond embryogenesis and its large size as fertilizable egg makes it a very convenient target for injecting trans genes, mRNAs, and anti-sense morpholinos for knockdown of gene expression. Modern technical developments in gene expression, transgenesis, and fluorescent reporters allows one to challenge zebrafish embryos with compounds, analyze gene expression changes in thousands of genes, and at the same time examine structural changes in the nervous system. Combining this information with gene manipula tion techniques followed by behavioral analysis allows one to relate specific environ mental changes with effects upon specific windows of development and neuronal targets. Fluorescent transgenes marking specific regions of the nervous system allow one to follow effects in close to real-time. Examples of using this combination of ap proaches will be presented using challenges with the organophosphate chlorpyrifos.

### 2025 BEHAVIORAL SCREENS FOR DETECTING DEVELOPMENTAL NEUROTOXICITY IN LARVAL ZEBRAFISH.

S. Padilla, Integrated Systems Toxicology Division, U.S. EPA, Research Triangle Park, NC.

As part of the EPA's effort to develop an in vivo, vertebrate screen for toxic chemi cals, we have characterized basic behaviors of 6-day post-fertilization (dpf) zebrafish (Danio rerio) larvae in a microtiter plate format. Our main goal is to develop a con venient, reproducible method for rapidly assessing abnormal nervous system func tion in larval zebrafish. Larvae were individually reared and tested in 96-well mi crotiter plates. A video tracking system and attendant software were used to record locomotor activity (measured as total distance traveled) under varying of light (visible light) and dark (infrared light) conditions. Control larvae exposed to neuroac tive drugs showed locomotor changes similar to those obtained in higher verte brates. Zebrafish exposed (0–5 dpf) to prototypic neurotoxic compounds showed altered activity at 6 dpf that were dose-related, whereas developmental exposure to non-neurotoxic compounds did not alter activity. We conclude that even very young zebrafish possess a rich behavioral repertoire, especially when studied for rel atively long periods of time (up to 1 hour), and under varying environmental con ditions. Furthermore, activity is perturbed by both acute drug exposure and develop mental exposure to neurotoxic compounds. This is an abstract of a proposed presentation and does not reflect Agency policy.

### 2026 A ZEBRAFISH MODEL FOR WHAT AILS, AND POSSIBLY CURES, US: A BEHAVIORAL PERSPECTIVE OF DEVELOPMENTAL LEAD AND MERCURY TOXICITY.

D. Weber1, J. Smith1, M. Wolter1, X. Xu1 and M. Carval1, 1University of Wisconsin-Milwaukee, Milwaukee, WI and 2Grand Valley State University, Allendale, MI.

Metal contaminants are neurotoxic to developing nervous systems in fetuses and children. Of concern are exposures to lead (Pb2+) due to associations with older urban environments and mercury (Hg2+ and methylmercury: MeHg); due to assoc iations with fish consumption. Both metals pass through the placental barrier and put the developing fetus at risk. Zebrafish were utilized to model developmental ex posures and human prospective studies due to short intergenerational periods. We developed sensory-startle response and learning assays for both short- and long term behavioral outcomes that are sensitive to toxic metal exposures. These changes were related to specific anatomical, physiological, and molecular changes. Finally, we investigated nutritional, therapeutic, or social/environmental enrichment inter ventions to reduce these deficits. Developmental metal exposures altered escape pa rameters in the startle response sequence: latency of reaction (longer), maximum escape velocity (slower) and duration of escape swim (longer). Reaction patterns to visual stimuli by adult fish developmentally exposed to metal contaminants were disrupt ed. Using various behavioral paradigms, learning was impaired in fish develop mentally exposed to Pb2+ or MeHg. Selenium may be important in mitigating spe cific neurobehavioral deficits due to MeHg exposure; Li+ is used to treat behaviors often associated with Pb2+ exposure in children. Co-exposures selelenomethionine mitigated changes due to developmental exposures with MeHg in larval and adult startle response behaviors but not learning. Similar outcomes in larvae and adults were observed with developmental co-exposures of Li+ and Pb2+. Changes in te lencephalon structure, retinal electrophysiology, and gene expression correlated with behavioral changes. These data indicate that developmental metal exposures cause short- and long-term behavioral abnormalities, are correlated to specific under lying mechanisms and parallel observations in humans and mammalian models.

### 2027 THE ZEBRAFISH AS A MODEL FOR DEVELOPMENTAL EXPOSURE TO PYRETHROID PESTICIDES.

D. A. White1, A. DeMicco1, K. R. Coopet1 and J. R. Richardson1, 1Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ and 2EOSHI, UMDNJ, Piscataway, NJ.

Pyrethroids are one of the most commonly used residential and agricultural insecti cides. Pyrethroid metabolites have been found in the urine of pregnant women and preschool children, raising concern that developmental pyrethroid exposure may cause damage to the developing nervous system. Data from the Richardson labora tory indicate that mice exposed during development to the pyrethroid deltamethrin exhibit elevated dopamine transporter (DAT) levels, hyperactivity and impulsive like behavior, at doses at or below the EPA-established no observable adverse effect level (NOAEL). We are using the zebrafish to test the hypothesis that developmen tal pyrethroid exposure alters dopaminergic neuronal development, and has a long term impact on gene expression and behavior. For our studies, we chose to dose at or below the NOAEL calculated from our behavior studies, and found two consistent phenotypes, body axis curvature and spasms, reminiscent of the classic syn dromes observed with pyrethroid toxicity. Treatment with Diazepam (50 µM) reduced spasms, while treatment with the sodium channel antagonist M5-222 reduced both spasms and body curvature, suggesting that pyrethroid-induced neu rotoxicity may be similar to the effects of developmental exposure to nicotine or pilocarpine, which are direct agonists of cholinergic receptors, which are indirectly stimulated by inhibition of acetylcholinesterase by OP pesticides like CPE. These studies demonstrate the value of the zebrafish model for studying pyrethroid neurotoxicity.

### 2028 PERSISTING EFFECTS OF EARLY DEVELOPMENTAL OP PESTICIDE EXPOSURE ON COGNITIVE AND SENSORIMOTOR FUNCTION.

E. D. Levin, Department of Psychiatry, Duke University Medical Center, Durham, NC.

Zebrafish can serve an important role as a supplementary model to high through put cell based assays and classic in vivo rodent models of developmental neurotoxi city. It has advantages over the behavior in vitro models and temporal development process in which to study neurotoxic events. It has advantages over rodent assays by having a clear chori on which permits continuous visual access to the developing brain. The development of sensitive and reliable zebrafish behav ioral assays in our lab and others provides critical functional information concern ing the adverse effects of developmental neurotoxicity. We have developed tests of learning and memory as well as sensorimotor startling response. In a series of studies we have determined the persisting neurobehavioral effects of early developmen tal exposure to the organophosphate pesticide, chlorpyrifos (CPF) in zebrafish. We have found that developmental CPF (100 ng/ml, days 1-5) exposure causes persist ent learning impairment in memory as measured by a spatial discrimination apparatus. The learning impairment was accompanied by a significant and persisting decrease in brain dopamine concentration. Developmental CPF exposure also significantly increased locomotor response to a tactile startle. This was similar to the effects of developmental exposure to nicotine or pilocarpine, which are direct agonists of cholinergic receptors, which are indirectly stimulated by inhibition of acetylcholinesterase by OP pesticides like CPE. These studies demonstrate the value of the zebrafish model for studying developmental neurobehavioral toxicology. Together with the large information bases being gathered using high throughput cell-based assays and the detailed neurobehavioral impairments being studied using the classic rodent assays, zebrafish can provide medium throughput mechanistic information with which to help knit together a more complete understanding of neurotoxic vulnerability.

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### 2029 HIGH-THROUGHPUT ELECTROPHYSIOLOGY: 21ST CENTURY TOXICITY TESTING APPROACHES WITH FUNCTIONAL OUTCOMES.

T. J. Shafer1 and G. Kirsch1, 1Neurotoxicology Division U.S. Environmental Protection Agency, Research Triangle Park, NC, and 2ChanTest, Cleveland, OH.

The NAS report on Toxicity Testing in the 21st Century envisions a future ap proach to toxicity testing that relies on in vitro, high-throughput approaches to identify and characterize toxicity hazards of environmental chemicals. These ap proaches are expected to replace or reduce the number of animals needed for toxicity testing. However, description of adverse effects for the purpose of hazard identifi cation has relied predominantly on changes in structure and/or function in
animals. In addition, many endpoints measured in high-throughput or high-content assays are biochemical and difficult to link directly to functional changes. For excitable tissues such as in the nervous and cardiac system, in vivo electrophysiological assessments have been widely and successfully utilized to describe adverse effects, whereas in vitro electrophysiological approaches have provided important information on mechanisms-of-actions of pesticides, metals, and other compounds. In recent years, new high-throughput/high-content electrophysiological assays have been developed and widely utilized for drug target screening and/or safety pharmacology—for example, screening of compound effects on hERG channels to identify those that produce torsades de pointes, a lethal cardiac side-effect of some drugs. Many of these electrophysiological approaches can be readily adapted to toxicity testing and will provide high-content information in addition to their throughput capabilities. Thus, these approaches can provide not only information on functional changes in electrically excitable tissues, but also information regarding potential toxicity pathways by which compounds may disrupt function. This session will describe a number of these HTS approaches for electrophysiology, and discuss their use for safety and toxicity testing. More importantly, it will focus on how these approaches can be further adapted for use in 21st century toxicity testing for toxicity pathway and hazard identification.

2030 USE OF CELL- AND TISSUE-BASED METHODS FOR RAPID IDENTIFICATION OF CARDIOVASCULAR TOXICITY PATHWAYS IN DISCOVERY TOXICOLOGY.


The cost impact of late-stage attrition of drug candidates or withdrawal of marketed drugs due to cardiovascular toxicity has motivated the pharmaceutical industry to implement a more proactive approach to cardiovascular risk assessment, particularly with respect to drug-induced proarrhythmia associated with delayed ventricular repolarization. Effective cardiovascular risk assessment should provide earlier evaluation of electrophysiologic and hemodynamic cardiovascular toxicities through thoughtful implementation of predictive in vitro, ex vivo and in vivo assays. The overarching goal is to identify target- or structure-based liabilities earlier so that optimized drug candidates less prone to failure can be identified and progressed into clinical development. For cardiovascular liability assays to influence early discovery effectively, they should have robust throughput, require low quantities of compounds, provide rapid results to drive decision-making and medicinal chemistry optimization; and offer flexibility based on project-specific needs that differ based on the toxicity and target/indication. An initial tier of high-throughput in vitro assays can be used for “toxicity profiling” and if a potential liability is identified within a chemical series or a lead compound, additional more robust assays can be used to provide additional risk perspective and distinguish toxic from non-toxic candidate compounds. These examples will highlight the utility of combining different assays with varying throughput and information content to identify and/or resolve toxicities.

2031 AUTOMATED PATCH CLAMP ASSESSMENT OF PYRETHROID INSECTICIDE INTERACTIONS WITH CLONED HUMAN NA⁺ CHANNELS.

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Automated patch clamp screens of recombinant, human ion channels provide rapid, cost-effective assessment of potential toxicity under conditions that preserve channel functionality and provide mechanistic insight. This technique is well established in cardiac safety assessments of pharmaceuticals, where hERG potassium channel inhibition is the most common cause of drug-induced cardiac arrhythmia. However, similar methods also may be appropriate in testing environmental toxicants that act on ion channels. As proof-of-concept, we have determined the effects of 11 pyrethroid insecticides against a panel of eight voltage-gated Na⁺ channels (stably expressed in CHO cells) in ScreenPatch™ assays using the IonWorks™ Quattro automated patch clamp system. By performing screening at a high concentration we were able to detect effects of pyrethroids on Na⁺ channel gating and separate the compounds into two major groups: compounds that slowed the time-course of Na⁺ channel inactivation (e.g., bioallethrin, permethrin), and compounds that produced a significant level of persistent sodium current (e.g., esfenvalerate, deltamethrin). hNav1.8/β3 channels showed the highest sensitivity to pyrethroids; 10 compounds of 11 total tested (10/11) were detected as positive. The rank order of sensitivity among other Na⁺ channels was: hNav1.2 (9/11) > hNav1.5 (9/11) > hNav1.6 (8/11) > hNav1.7 (7/11) > hNav1.1 (6/11) > hNav1.4 (6/11) > hNav1.3 (4/11). Our results demonstrate that an automated Na⁺ Channel Panel™ assay can provide highly sensitive functional screening to detect potential toxicity of pyrethroids and other compounds that interact with Na⁺ channels. High-throughput electrophysiological recording facilitates large-scale screening of multiple ion channel targets that could be useful in early-stage toxicity testing. The advantages and limitations of this approach will be discussed.

2032 THE PROMISE OF MICROELECTRODE ARRAY APPROACHES FOR TOXICITY TESTING: EXAMPLES WITH NEUROACTIVE CHEMICALS.

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While high-throughput patch clamping formats provide rapid characterization of chemical effects on ion channel function and kinetics, the limitations of such systems often include the need for channel by channel characterization, requirements for transfected, rather than primary cells, and the inability to assess effects on spatial and temporal functions of networks of cells. Microelectrode Arrays (MEAs) allow recordings from primary neurons or cardiomyocytes, and provide information derived from individual sites as well as spatial and temporal information. Because the function of the cell rather than a specific ion channel is measured, drug or chemical actions at any site affecting electrical activity of the cell may be detected, an advantage when screening chemicals where no information exists regarding the type of mode of action. The pattern of activity allows for information regarding the site of action (i.e. channel) for a chemical. MEAs have been used to examine a wide variety of drugs and chemicals, including flumoxetine (a selective 5-HT reuptake inhibitor), muscimol (a GABA, R agonist), and verapamil (a calcium channel blocker) as part of a multi-lab study to determine reproducibility of data across labs and MEA platforms. These examples will be used to discuss both the advantages as well as the limitations of MEA approaches for predictive toxicity testing. (This abstract does not reflect EPA policy).

2033 FDA USE OF DATA FROM IN VITRO ELECTROPHYSIOLOGY IN A REGULATORY ENVIRONMENT: APPLYING LESSONS LEARNED IN SAFETY PHARMACOLOGY TO TOXICITY TESTING.


New drugs are routinely evaluated for adverse cardiovascular effects in dedicated safety pharmacology studies. As a component of this evaluation, drugs are tested in an in vitro electrophysiology assay for effects on an ionic current, human either a go-go related gene (hERG) current, since inhibition of this current is the most common mechanism for drug-induced QT prolongation and torsade de pointes (Tdp). The concordance of hERG current inhibition to QT prolongation and Tdp in patients has been established for several drugs labeled for or removed from the U.S. market for this reason. FDA considers hERG inhibitory potency in the risk assessment and requests a concurrent positive control to account for sensitivity differences across studies and laboratories. Tests for drug effects on additional ionic currents, e.g., sodium and calcium currents, have also proven useful in evaluating cardiac safety of selected drugs. FDA uses in vitro current data in the integrated risk assessment of new drugs, which then informs further in vivo nonclinical testing and clinical trials. Sponsors additionally use in vitro data for drug candidate selection. One liability of such an approach is the potential loss of safe drugs based on in vitro data. This example demonstrates that in vitro electrophysiology data is useful in risk assessment when relevant toxicity mechanisms are identified and concordance of in vitro to in vivo and clinical data is demonstrated.

2034 MINERALS AND METALS: PROS AND CONS OF DELIBERATE EXPOSURE.

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Metals and minerals are known to induce adverse effects ranging from oxidative stress to carcinogenesis. However, some are administered or are under consideration for therapeutic intent either as direct administration or as a consequence of metal-
to-metal joint replacement. For example, oral iron can reduce the incidence of anemia—number 11 on the World Health Organization's top 20 list of global disease burden. On the other hand, there is a risk of increasing infection and morbidity/mortality in developing countries or risk of chronic subclinical inflammation and colon cancer in the developed world. We will explore the potential of metals and minerals in therapy and in parallel will consider the potential adverse effects that need to be considered. The presentations are designed to provide attendees with important and biologically relevant issues related to in vivo exposures to metals and minerals, a comprehensive and stimulating state-of-the-art update on innovative testing methods, and a vision of expected scientific advances in the understanding of how minerals and metals can affect developmental, degenerative, and carcinogenic processes. The focus of this session encompasses concepts and themes from cell physiology through to molecular biology with an overall goal of ensuring a better understanding of the assessment of hazard and risk associated with exposures to minerals and metals. As such, it is intended for basic and applied scientists in academia, government, and industry.

W 2035 THERAPEUTIC USES OF METALS AND MINERALS: THE RISK-BENEFIT INTERFACE.

J. Powell, Department of Micronutrient Status Research, MRC Institute for Human Nutrition Research, Cambridge, United Kingdom.

There is widespread usage of inorganic-based therapeutics from soluble salts and metal-based complexes through to nano engineered structures and solid implants. The chemical composition and physical form of these metal based materials present widely different challenges in understanding the risk/benefit ratio. For example, iron for supplemental use is mainly in the ferrous (Fe(II)) form as a simple salt. Although well absorbed, mucosal damage through facile redox cycling results in adverse intestinal effects or, potentially, exacerbation of inflammation and an enhancement of colon cancer risk. Ferric iron (Fe(III)) is less prone to initiation of redox cycling but absorption is poor due to intestinal luminal hydrolysis. Attempts to circumvent poor absorption involve iron chelation or nanoparticulate formulation for slow release but toxicity of the chelator or the nanoparticles must be established. For nanoparticles, the possibility of endocytic uptake means that particle size, surface characteristics, persistence and chemical composition are key in determining the cellular utility versus toxicity. Thus, oral iron supplementation illustrates many of the risk/benefit considerations in inorganic-based therapeutics since physico-chemical form determines cellular uptake and utilisation as well as both intra- and extra-cellular toxicities. Key determinants of physico-chemical form are solubility/persistence, redox state, chemical speciation, size and surface characteristics. Recently we have started to apply these principles to the effects of wear debris to metal-on-metal hip replacements. Both nanoparticulate and soluble cobalt and chromium species have been identified, while carcinogenic Cr(VI) is absent and Cr(III) appears to be the only chromium species. An increased risk of lymphophaenia has been identified in patients and we are examining which of the Co(II)/Cr(III) forms leads to these effects.

W 2036 PROTECTION AGAINST CHROMIUM (VI)-INDUCED OXIDATIVE STRESS AND APOPTOSIS BY NRF2.

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Nrf2, an antioxidant-activated cap ‘n’ collar bZip transcription factor, has recently emerged as a critical regulator of cellular defense against oxidative stress, a prominent component in the pathogenesis of a broad range of disease and chemical toxicity including metal-induced cancer and toxicity. Chromium (Cr) is a major environmental/occupational toxic metal and an established human carcinogen. Exposure to Cr(VI) elicits respiratory cancer and multi-organ toxicity including renal tubular injury, subrenal fibrosis, allergy, and asthma in humans. At a cellular level, Cr(VI) induces prominent oxidative stress and apoptosis. Data demonstrated that Nrf2 is critically involved in the cellular response to Cr(VI)-induced ROS production and cell death. Cr(VI) at a non-toxic concentration activates Nrf2 by interacting with critical cysteine residues of Nrf2 and Keap1—a cytoplasmic Nrf2-binding protein that controls the degradation of Nrf2 in the absence of an inducer. Activated Nrf2 mediates induction of a battery of cytoprotective genes encoding phase 2 detoxification enzymes and antioxidative proteins that inhibit ROS production and apoptosis by Cr(VI). Targeted disruption or siRNA-mediated knockdown of Nrf2 markedly elevated ROS production under basal conditions and in the presence of Cr(VI) that involves altered oxygen and energy metabolism in the mitochondria. Nrf2 potentially coordinate a network of protective mechanisms against mitochondrial damage by toxic metals. In summary, Nrf2 protects against chromium (VI)-induced apoptosis via a mechanism that may also be applicable to other metals such as arsenic and cadmium.

W 2037 FUNCTIONAL PROFILING TO IDENTIFY METAL TOXICITY PATHWAYS IN YEAST.

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We have used a chemical genomics approach to identify the genes that modulate the cellular toxicity of copper, iron, cadmium and arsenicals in the yeast Saccharomyces cerevisiae. Our results indicate the presence of two different detoxification pathways for iron and copper that converge toward the vacuole. The product of several of the identified genes in these pathways form molecular complexes that are conserved in mammals and include the retnor, endosomal sorting complex required for transport (ESCRT) and AP-3 complexes. Functional profiles provided evidence of the requirement for highly-conserved biological processes in the response against both arsenicals including tubulin folding, DNA double-strand break repair and chromatin modification. Of particular interest, we found that the SAS2 gene encoding the catalytic subunit of the heterotrimetric something about silencing (SAS) complex responsible for the acetylation of histone 4 at lysine 16 (H4K16) is required for optimal growth of yeast in the presence of arsenite (AsIII). We demonstrated that H4K16 acetylation is necessary for yeast's resistance to AsIII through modulation of chromatin state. Analysis of strains sensitive to cadmium identified not unexpectedly key roles for vacuolar transport and homeostasis of multiple metals as with iron. Surprisingly, oxidative stress was not implicated while chromatin modification and lipid metabolism were identified as key processes for cadmium sensitivity. Together these studies have provided insight into the conserved molecular processes involved in metal homeostasis.

W 2038 METALS AND OXIDATIVE IMPAIRMENT IN NEURODEGENERATIVE DISORDERS.

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Chronic exposure to manganese (Mn) leads to a movement disorder due to preferential Mn accumulation in the basal ganglia. Iron (Fe) deficiency also results in increased brain Mn levels. To address whether brain Mn distribution differs in paradigms of increased Mn exposure vs. Fe deficiency, we utilized 4 experimental populations. Rats on different diets (Mn-treated [MnT], Fe deficient [FeD], or Fe supplemented [FeS]) were given weekly intravenous Mn injections for 6-14 weeks; control (CN) rats were fed a control diet and received sterile saline injections. Brain Mn levels were measured by atomic absorption spectrophotometry and magnetic resonance imaging (MRI). As a novel approach for exploring dopaminergic (DAergic) neurodegeneration in vivo, we used pharmacological magnetic resonance imaging (phMRI) to assess the blood oxygenation level-dependent (BOLD) response to amphetamine (AMPH). Changes in dietary Fe levels (either increased or decreased) resulted in regionally specific increases in brain Mn levels compared with controls. Furthermore, there was no difference in either Fe or Mn accumulation between FeS or FeD animals. Additionally, Mn treatments were associated with increased isoprostane levels, a marker of oxidative stress. Increased uptake of Mn in the brain was shown to coincide with upregulation of both the divalent metal transporter (DMT-1) and transferrin. Finally, relative to controls, Mn-treated rats exhibited a widespread reduction in the AMPH-induced BOLD response throughout the basal ganglia. Our data suggest that despite its essentiality, vulnerable FeD populations exposed to high levels of Mn may be at risk for potentially dangerous alterations in brain metal levels and function. (Supported by NIEHS 10563 & DoD W81XWH-05-1-0339)
century include high-throughput screening technologies to identify cellular interactions with NMs for efficacy and safety. This session will present ongoing genomics, proteomics, and metabolomics studies of interactions between natural and engineered NMs and biological systems. Findings of novel interactions of NMs and biological systems will be highlighted and the feasibility of these approaches for future comprehensive studies of NM efficacy and safety will be discussed. Examples of in vitro cellular interactions with a variety of NMs will be provided which include mRNA, miRNA, and proteomic expression profiles of human and mammalian cells exposed to nanotubes, nanocrystals (quantum dots), dendrimers and nano-scale particles of both terrestrial and extra-terrestrial origin. Selective activation of specific genes, proteins, and cellular signaling pathways will be related to possible mechanisms of action. We will highlight how the addition of metabolomics to other omics based studies can further define the effects of NMs on biological systems after environmental exposures. The final presentation will expand upon the systems biology approach and show how multiple ‘omics technologies can provide mechanistic meaning when individual data sets are analyzed ranging from a global to subcellular view. This session should be of interest to all investigators interested in state-of-the-art ‘omics technologies for screening the effects of foreign materials, including NMs, in humans and other organisms.

W 2040 MESSENGER RNA AND MICRONORNA EXPRESSION PROFILING OF NANOMATERIAL INTERACTIONS WITH PRIMARY HUMAN SKIN AND LUNG CELLS.

M. Cunningham, Nanomics Biosciences, Inc., Cary, NC.

A variety of nanomaterials (e.g. carbon nanotubes, dendrimers, lunar dust, quantum dots, nano- and low micron-scale particulates) were exposed to primary human skin, lung, and neuronal cells in vitro. The activities of mRNAs and miRNAs were profiled in an effort to elucidate tissue-specific responses to various NMs. The data were analyzed and compared between the different cell types and materials. Results highlighting the selective activation of unique sub-cellular signaling pathways in different cell types representing different exposure routes and the consequences of NM exposure will be presented.

W 2041 CARBON NANOPARTICLE EXPOSURE ALTERS BARRIER EPITHELIAL CELL FUNCTION: PROTEOMIC AND ELECTROPHYSIOLOGICAL ANALYSES.

F. A. Witzmann, Department of Cellular & Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN.

Murine kidney barrier cells (mmpCCDcH) were exposed to various concentrations of fullerenes, single-walled carbon nanotubes, and unrefined and functionalized multi-walled carbon nanotubes, in vitro. Differential protein expression was assessed independently by label-free quantitative mass spectrometry and 2DE. Transepithelial electrical resistance and hormone stimulated transport were also measured as physiological endpoints. Results highlighting exposure-related alterations in protein expression including cell adhesion and tight-junctional proteins, and barrier cell function unique to nanoparticle type, dose, and degree of functionalization will be presented.

W 2042 GENOMIC SIGNATURES FOR SIZE-DEPENDENT BIOLOGICAL EFFECTS OF GOLD NANOPARTICLES.


Nanoparticles are used increasingly in consumer products and biomedical applications, yet the cellular interaction mechanism is not well understood for nanomaterials of different physico-chemical properties. Gold nanoparticles (Au-NPs) are used as the model system to help understand the size-dependent biological effects of nanoparticles. Cells treated with Au-NPs ranging from 2 nm to 280 nm were studied. Whole genome expression measurements indicate size-dependent effects at the molecular level. Gene function, promoter and pathway analyses reveal differential signaling processes that are correlated with nanoparticle sizes.

W 2043 IN VITRO AND IN VIVO METABOLICOM AND PROTEOMIC BIOMARKER STUDIES OF III-V SEMICONDUCTORS ON RENAL PROXIMAL TUBULE CELLS.

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Recent studies from a number of laboratories have demonstrated size-dependent glomerular clearance of nanoparticles of various compositions with vascular accumulation in several renal cell types and subsequent cellular toxicity. Nanoparticles of the III-V semiconductors gallium arsenide (GaAs) and indium arsenide (InAs) are being used for a variety of microelectronic and imaging applications but the toxicological database on these materials is limited. In order to provide basic scientific information on cellular responses to GaAs and InAs particles, a series of rodent studies using intracheally administered 4-5 micron particles of GaAs or InAs were conducted to evaluate in vivo proteomic (protein expression patterns) and metabolomic (heme pathway) responses to these agents in renal tubule cells. Complimentary in vitro studies using primary cultures of renal proximal tubule cells from both rodents and humans were conducted to examine molecular responses of this target cell population to soluble Ga, In, and As compounds alone or as combinations. Metabolomic and proteomic biomarker endpoints were monitored to assess early cellular responses to these agents and manifestations of cell injury. Overall, results of these combined in vivo and in vitro studies demonstrated both element/composition-specific heme pathway and proteomic biomarker patterns and the importance of gender in mediating observed cellular responses. Oxidative stress and stress protein response patterns appear to be major factors in mediating the cellular toxicity of these agents in renal tubule cell populations.

W 2044 DYNAMIC NETWORK ANALYSIS OF NANOSILICA-INDUCED TOXICITY PATHWAYS USING MICROARRAY AND PROTEOMIC DATA.

K. Waters. Computational Biology & Bioinformatics, Pacific Northwest National Laboratory, Richland, WA. Sponsor: M. Cunningham.

Nanosilica exposures to mouse macrophage and alveolar epithelial cells were used to investigate and determine the modes of action for nanomaterial-induced inflammation and cytotoxicity in the lung. Microarray studies in cells exposed to nano-sized amorphous silica demonstrated time- and dose-dependent regulation of biological pathways that can be correlated to cytotoxicity endpoints. Global proteomics analysis of secreted proteins identified extracellular factors that are known to modulate the inflammatory response. Network inference analyses determined critical control points in the response pathways that have subsequently validated as determinates of responses to nanosilica using siRNA and pharmacological knockdown studies in cells. Response biomarkers for silica cytotoxicity pathways provide a foundation for dose-response modeling of nanosilica biocompatibility.

W 2045 THE PROCESS OF DEFINING RISK FOR ENVIRONMENTAL CHEMICALS HAVING SIGNIFICANT SKIN EXPOSURE AND ABSORPTION POTENTIAL.

W. G. Reifenrath1 and I. H. Ross2, 1Stratocor, Inc., Richmond, CA and 2Risk Sciences, LLC, Sacramento, CA.

Skin exposure and subsequent absorption of environmental contaminants are often critical issues for regulatory decisions concerning NM treatment or remediation at hazardous waste sites. Likewise, these issues are important in the registration or re-registration of pesticides. To address these points, laboratory studies are generally conducted with excised skin or animal models to determine the extent (percent absorption) or rate of penetration (permeability constant) of a chemical in question. In addition, exposure determinations, often based on field studies, determine the form and amount of chemical that can potentially reach the skin. Biomonitoring studies can integrate the processes of skin exposure and systemic absorption. To address these points, laboratory studies are generally conducted with excised skin or animal models to determine the extent (percent absorption) or rate of penetration (permeability constant) of a chemical in question. In addition, exposure determinations, often based on field studies, determine the form and amount of chemical that can potentially reach the skin. Biomonitoring studies can integrate the processes of skin exposure and systemic absorption. The forgoing studies generate numbers, which require a translation into the potential for bioeffect, and the significance of that effect, which leads to a risk assessment. Regulators, such as the U.S. EPA, then make decisions based on the assembled data. The process works best when there is communication between all parties, starting with the design of experimental protocols. In recent years, there has been an increasing reliance on in vitro permeation data. While test guidelines are available for percutaneous absorption, actual studies have unique aspects that need
to be addressed or negotiated. Decisions on seemingly small details at any level can ultimately have major impacts. Therefore, it is our intent to give a vertical perspective on the process by which safety assessments are made, starting at the laboratory and ending with a regulatory decision, and highlighting those aspects that can shape the outcome.

2046 DERMAL ABSORPTION IN THE EXPOSURE ASSESSMENT PROCESS.
J. H. Ross, Risk Sciences LLC, Sacramento, CA.

In the absence of definitive data, the regulatory default for dermal absorption is 100%. Since potential exposure to all but the highest volatility pesticides (e.g., fugitives) is overwhelmingly dermal in many scenarios, it makes the measurement of dermal absorption arguably the single most important factor in defining absorbed dose. Because risk assessments are supposed to be for humans rather than rats, and over a wide range of chemical types humans absorb on average 5-fold less than rats through the skin, it is critical to be able to measure absorption in humans. However, conducting in vivo absorption studies in humans has become virtually impossible because of human subjects regulations created by EPA in 2006. Thus, acceptable alternatives to estimating human absorption accurately are necessary. A superficial effort to validate the use of in vitro human dermal absorption data as a predictor of in vivo human absorption has been made at the time OECD adapted its in vitro guidelines in 1996. A validation method is proposed and specific examples of the “parallelogram method” are discussed with historical context including permethrin, fluazifop butyl, and piperylon butoxide.

2047 TRANSLATING IN VITRO SKIN ABSORPTION DATA INTO ESTIMATES OF HUMAN SKIN ABSORPTION.
W. G. Reifenrath, Stratacon, Inc., Richmond, CA.

A significant limitation of in vitro skin diffusion studies has often been stated as the lack of dermal microcirculation in the upper layers of the dermis (papillary dermis). As a result, in vitro percutaneous absorption determinations based only on penetration into the receptor fluid may underestimate in vivo skin absorption, particularly for lipophilic test compounds, because of incomplete partitioning of penetrant into aqueous receptor fluids. Opinions and test guidelines vary regarding whether to include skin residues as percutaneous absorption and even the definition of skin residue. Dermis accumulations in vitro likely represent compound that normally (in vivo) would have left the skin tissue, so it makes sense to include dermal levels as likely to have been absorbed if the dermal microcirculation was functioning. The best fit of in vitro data to parallel in vivo data was the inclusion of dermis and receptor fluid values and not to include epidermis or tape strip residues. For 4 species and organic compounds spanning a wide range of physical properties, there was good agreement between in vivo vs in vivo percutaneous absorption when in vitro dermis residues were included with those in receptor fluid.

2048 IN VITRO DERMAL ABSORPTION OF SOIL CONTAMINANTS: IMPORTANCE OF MODELING FIELD EXPOSURE CONDITIONS.

The importance of evolving field and laboratory methods in determining dermal absorption of environmental contaminants is rarely considered in human health hazard/risk assessment, however, the advantages and disadvantages of these methods need to be clearly understood, especially as to their ability to model field conditions. Health Canada dermal absorption research has evolved from early in vivo tests with animals such as rhesus monkeys, to in vitro test with excised fresh viable human skin tissue. Other alternatives to animal testing such as in silico computer models are also in development. Recently, our laboratory has commenced testing radiolabeled soil contaminants (e.g. 14C-benz[a]pyrene, 14C-nonyl phenol, mercury203 and nickel63) in vitro employing an aqueous suspension of commercial gardening soil. This wet soil application may best model swimming/bathing exposure and perhaps can be extrapolated to children playing in mud, and possibly to soil particles on skin wet from eccrine sweat, but tests are needed with actual field soils from contaminated sites in Canada applied to skin under other more commonly encountered exposure conditions. The importance of using relevant field exposure conditions is fundamental to reliable extrapolations of in vitro data for health risk assessment purposes.

2049 SURFACE DEPOSITION OF PESTICIDES AND DERMAL ABSORPTION OF RESIDUES IN AGRICULTURAL AND URBAN ENVIRONMENTS, DETERMINANTS OF PESTICIDE ABSORPTION IN AGRICULTURAL AND RESIDENTIAL SETTINGS.
R. I. Krieger, Z. Chen, G. Sankaran and W. Song, Department of Entomology, University of California, Riverside, Riverside, CA.

Pesticide application inevitably produces a potential inhalation exposure of applicators and others in agricultural and residential environments. The more telling unintended (or unavoidable) human exposures that follow are derived from surface deposition of ug/cm2 residues. Contact transfer of these residues indoors or in agricultural settings produce ug/kg bw-d exposures that occur directly or indirectly from the chemical. Human exposures are best measured using urine biomarkers under normal conditions of pesticide use. Chlorpyrifos, 2, 4-D and triclopyr, and malathion exposures associated with dermal exposure have been measured. Aspects of these studies illustrate the importance of detailed knowledge of pattern of pesticide use, description of the environmental fate of pesticides and their residues, and an understanding of the disposition of the chemicals in people. Interpretation of the results requires full appreciation of the importance of dermal exposure and absorption for predictive and responsible exposure assessment and risk characterization.

2050 REGULATORY PERSPECTIVES ON THE USE OF DERMAL ABSORPTION DATA FOR RISK ASSESSMENT.

In 1992, the Office of Health and Environmental Assessment of the U.S. EPA issued an interim report, Dermal Exposure Assessment: Principles and Applications, to provide guidance for conducting dermal exposure assessments. The 2001 Superfund RAGS Part E, Interim Supplemental Guidance for Dermal Risk Assessments, was the result of Superfund Dermal Workgroup meetings on issues associated with the characterization of risk resulting from dermal exposures at Superfund sites, which was of limited relevance to pesticides. The Office of Pesticide Programs (OPP) has relied upon OPPTS 870.7600 guideline dermal absorption studies in rats under conditions that mimic occupational exposures in order to predict dermal absorption of pesticides in humans. Because human skin is usually less permeable than rat skin, there is increasing interest in using human skin to refine the dermal absorption factor used for risk assessment. In vitro studies using human skin provide an alternative to human studies for this purpose. However, in vitro studies are currently not accepted by OPP as a stand-alone to predict human dermal absorption factors because in vitro methods have not been standardized or validated. The Triple Pack, or Parallelogram, approach allows the predictivity of the in vitro assay to be assessed by running an in vivo study (in rats) and an in vitro study (using rat skin and human skin) under similar conditions (e.g. same formulations, doses, times, and treatment of the skin with regard to washing) to assess the predictivity of the in vitro method. In an ideal case, the ratio of dermal absorption in rats in vivo and in vitro (the rat in vivo/rat in vitro ratio) is 1. After quantifying the predictivity of the in vitro method for rats, the in vitro results for human skin may be used to refine the dermal absorption factor. The ultimate goal is to obtain a database of Triple Pack studies amenable to analysis that may support using in vitro studies with human skin as a stand-alone for assessing the dermal absorption of pesticides.

2051 EU/OECD GUIDELINES, A PERSPECTIVE FROM BAYER CROPSCIENCE.

As a producer of novel plant protection products, Bayer CropScience needs to produce data on the dermal absorption of its active ingredients in order to refine operator, worker, bystander and resident risk assessments. These dermal absorption studies are performed according to the OECD guidelines and guidance document and also the EC guidance document. There are currently on-going projects to revise the EC/SANCO/222 guidance document and to provide a new OECD Guidance Notes document. This presentation will provide an update on the progress being
made with these projects and an outline of the Bayer CropScience approach to performing and interpreting dermal absorption studies. Examples will be given of areas where expert interpretation of the data from both in vitro and in vivo studies can differ. These will include the use of tape-stripping data, the determination of the end of absorption for in vivo studies and the use of "read across" between different formulation types.

**2052 TRANSLATION OF NONCLINICAL MODELS TO CLINICAL RISK MANAGEMENT STRATEGIES OF SEVERE INFECTION DISEASES WITH IMMUNOMODULATORY DRUGS.**

J. Kawabata and K. Komosar.

The recent development of immunomodulatory drugs to treat autoimmune and inflammatory diseases has resulted in significant patient benefit. However, modulation of immune responses may also result in decreased host resistance mechanisms and increased risk for infectious diseases and cancer. Some infrequent, but severe infectious diseases such as Progressive Multi-Focal Leukencephalopathy (PML) and tuberculosis, have had significant impact on public health and the development of immunomodulatory therapies. The mechanism for the development of these diseases with drugs of different mechanisms of action and the susceptibility factors of certain patients is not clear. Currently, knowledge of the mechanism-of-action of the drug being developed, findings from standard toxicology studies and studies of immune function assessment may help determine potential risk for PML or tuberculosis, but do not provide decision making information on relative risk in the early stages of drug development. Given the low incidence of these severe infectious diseases and strict inclusion criteria of clinical trials, it is difficult to determine if severe infections will be a risk until larger populations are treated. Moreover, clinical monitoring to assess changes in immune function that may lead to decreased host resistance or biomarkers of recurrence of microbes have not been adequately developed and validated. Application of approaches used in infection monitoring/prevention in the setting of clinical transplantation may provide some additional insight for the development of less suppressive immunomodulators. To address these significant gaps, research across many disciplines is needed to better predict and risk manage infectious diseases. The goal of this session is to increase awareness of this issue and stimulate discussion on approaches to develop translatable nonclinical and clinical assays/biomarkers for better risk assessment and management of infection liability in the clinic.

**2053 CLINICAL CONSEQUENCES OF ADVERSE UNINTENDED IMMUNOMODULATION.**


PML and reactivation of TB are the best known examples of increased risk of infection with newer immunomodulatory agents. This concern has been heightened recently by the continued reports of cases of PML with natalizumab, and by those occurring with other immunomodulators, namely rituximab for autoimmune diseases such as SLE, and alefacept for psoriasis. Assessment of increased risk for infection or malignancy in early clinical development is made more challenging by the small numbers of subjects enrolled in such trials, and by the strict entry criteria that typically exclude patients at higher risk of infection. In later development as larger numbers of subjects are exposed to drug, it may be possible to assess infection rate versus placebo populations. In addition to using a purely statistical approach, there is benefit to include a robust microbiological assessment of significant infections to tease out the potential of the drug to cause unusual opportunistic infection. This may or may not have been predicted by the mechanism of action. Importantly, there is a role for an assessment of immunotoxic potential based on a translational approach from nonclinical work. While no formal guidance for clinical immune function assessment currently exists, many approaches can be considered based on mechanism of action, populations to be studied, and data from immunotoxicology studies in animals. For example a TDAR approach is now widely used in clinical development and examples of its use both as a holistic indicator of immunosuppression and of a direct indicator of a drug effect on vaccination effectiveness will be discussed. Finally, looking to the future, what emerging technologies may help us to move beyond our assessment of risk for unintended consequences with immunomodulatory drugs, and our ability to identify and screen out patients at increased risk of these complications. As with other disciplines, personalyzed medicine may play an important role in the field of clinical immunotoxicity, helping us to bring the right drugs to the right patients.

**2054 RISK MANAGEMENT OF INFECTIONS WITH TRANSPANTATION.**

C. Kotton. Transplant and Immunocompromised Host Infectious Diseases, Infectious Diseases Division, Massachusetts General Hospital, Boston, MA. Sponsor: W. Komosar.

Immunomodulated hosts are at increased risk for both routine and opportunistic infections. Their "net state of immunosuppression" is determined by multiple components, including their immunosuppressive regimen, underlying disease state(s) (i.e. lupus, rheumatoid arthritis, inflammatory bowel disease), as well as other concomitant disease(s) (i.e. diabetes, renal insufficiency, heart failure). Different mechanisms of action of immunosuppressive agents may allow for prediction of the more likely infections, however, use of multiple agents for immunosuppression may confound our ability to anticipate certain infections. In addition, immunosuppressed hosts may develop previously unexpected infections. Clinical trials sometimes expose the risk of infection, although risk for many infections becomes unmasked during the post-marketing phase, especially for diseases with lower prevalence. The advent of molecular diagnostics has allowed for better screening and monitoring of immunocompromised hosts. Serologic assays generally have reduced sensitivity in this population that is much less likely to have a humoral response to infection.) The management of cytomegalovirus after solid organ transplant is an excellent example of an infection that has benefited from appropriate monitoring, prophylaxis, and treatment. Patient-specific diseases, such as reactivation Chagas disease or hepatitis B, can be also closely monitored and treated, greatly enhancing safety and clinical outcomes. These paradigms could be readily applied to clinical trials and post-marketing management of patients on immunosuppressive regimens and will be discussed further.

**2055 PROGRESSIVE MULTIFOCAL LEUKENCEPHALOPATHY AND IMMUNOMODULATORY DRUGS.**

T. Kawabata, Pfizer, Groton, CT.

Progressive Multifocal Leukeencephalopathy (PML) is a rare demylinating disease caused by JC virus infection and destruction of oligodendrocytes in the CNS. This disease has a high mortality rate and those that survive have permanent neurological problems. JC virus is a latent virus that is present in the kidneys, lymphocytes and bone marrow of most adults. With immunosuppression, primarily decreases in T cells, JC virus is reactivated, replicates and infects the CNS. In the past, PML has been primarily associated with HIV AIDS and hematologic malignancies. More recently, a low incidence of PML has also been found with the immunomodulatory agents natalizumab (Tysabri®), efalizumab (Raptiva®) and rituximab (Rituxan®). Due to risk benefit concerns, the clinical use of natalizumab and efalizumab have been significantly impacted. Natalizumab was withdrawn from the market in 2005 due to 3 cases of PML and re-introduced with a program that controls its clinical use and carefully monitors safety. Efalizumab was withdrawn from the market in early 2009 due to 4 cases of PML. For the toxicologist, methods to predict for the potential for JC virus reactivation and PML with drugs in development and approaches for risk management are not available. This is attributed to the limited understanding of the mechanisms JC virus reactivation with immunomodulation, PML pathogenesis and patient-related risk factors. Research to address these questions are hindered by the lack of a relevant animal model and low incidence rate in humans. This presentation will provide an overview of what is known about the pathogenesis of PML and immune mechanisms against JC virus reactivation and discuss the research gaps and opportunities.

**2056 MYCOBACTERIUM TUBERCULOSIS OVERVIEW AND THE REACTIVATION OF LATENT TUBERCULOSIS.**

W. J. Komosar, Autoimmune Disease Platform, Eli Lilly & Company, Indianapolis, IN.

Mycobacterium tuberculosis (MTb) is an intracellular, gram positive and acid-fast bacillus that is the etiologic agent of tuberculosis. Approximately one third of the world’s population is infected with MTb and tuberculosis is the leading cause of death associated with infectious diseases. The importance of a well functioning immune system is paramount in the individual’s ability to control and contain MTb through granuloma formation around the bacilli and intracellular killing of the bacilli. Through these immune mechanisms, the infection is controlled and exists in a latent, inactive state in most individuals. The development of immunomodulatory agents that selectively target immune components and/or functions must be evaluated for their potential to increase infection susceptibility. The exclusion of patients with active TB and possible latent TB infection (LTBI) is routinely required before initiating most biologic therapies for autoimmune diseases in clinical trials.
especially with anti-TNF therapies. Preclinical models of active tuberculosis infec-
tion in the mouse, rabbit and non-human primate provide unique opportunities to understand disease pathogenesis and assess risk in the development of new im-
munomodulatory agents. In some cases, these models recapitulate human tubercu-
losis pathogenesis and also can provide insight into the reactivation potential of LTBI, before larger populations of at risk patients are exposed in clinical trials. Each model will be presented and discussion of their use during clinical development will be introduced.

2057 THE USE OF NONCLINICAL ASSESSMENT STRATEGIES IN THE PREDICTION OF CLINICAL RISKS OF IMMUNOMODULATORY MOLECULES: CASE STUDY FOR ABATACEPT, A SELECTIVE CO-STIMULATION MODULATOR.

H. G. Haggerty, Drug Safety Evaluation, Bristol-Myers Squibb, Syracuse, NY.

While immunosuppressant molecules may protect against the adverse conse-
quences of their diseases or conditions, they also have the potential to affect how the body protects itself against infection and oncogenesis by impairing immunour-
seillance. There are a number of assessment strategies that can be undertaken to un-
derstand the effect of such molecules on immunocompetence, including host-re-
sistance and antigen-response models. Selection of appropriate assessment strategies must include an understanding of the mechanism of immunomodulation of the test molecule and the intended patient population for its therapeutic use, allowing for the prudent use of models that may provide the strongest relevance to identify-
potential risks in patients. This presentation will share some of the approaches used to evaluate the potential immunotoxocities of one novel immunotherapeutic protein and how those findings translated to the clinic. Abatacept selectively mod-
ulates T-cell activation by modulating the interaction of CD28 on T-cells with CD80/86 on antigen presenting cells and is marketed for treatment of rheumatoid arthritis. The safety of abatacept has been well characterized in a comprehensive program based on an understanding of the pathmechanism of abatacept and how it impacts the various components of the immune system, po-
tential safety issues were identified and special endpoints that evaluated the im-
mune system and its potential for adverse consequences included in nonclinical studies. In addition, the effect of abatacept or murine CTLA4Ig on the host im-
mune response to different infectious agents including, Pneumocystis carinii; murine cytomegalovirus; herpes simplex virus; and tuberculosis was evaluated. Although these types of infections are uncommon, or rare, the clinical data appear to correlate with the host resistance models. Thus, by understanding the mecha-
nism of action and intended patient population, the preclinical models evaluated were predictive of clinical outcomes.

2058 CYTOCHROME P4501A1 (CYP1A1) IS REQUIRED TO MEDIATE VASCULAR DYSFUNCTION, REACTIVE OXYGEN SPECIES, AND HYPERTENSION INDUCED BY 2, 3, 7, 8- TETRACHLORIDIBENZO-P-DIOXIN (TCDD).

P.G. Konie, L. N. Alghani, J. Scott and M. K. Walker, Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.

High dietary fat intake increases the risk of hypertension. Dietary fat intake also represents the major route of exposure to halogenated aromatic hydrocarbon pollu-
tants, which include halogenated dibeno-p-dioxins, dibenzofurans, and biphenyls. Data from the National Health and Nutrition Examination Survey (NHANES) link exposure to these pollutants to hypertension in the general U.S. population. Further, the link with hypertension remains even after controlling for age and body mass index. The halogenated aromatic hydrocarbons that are associated with hyper-
tension activate the aryl hydrocarbon receptor (AhR). Nevertheless, the mecha-
nism linking these environmental pollutants to hypertension is unknown. We tested the hypothesis that exposure to TCDD induces reactive oxygen species, vas-
cular dysfunction, and hypertension via a mechanism requiring cytochrome P4501A1 (CYP1A1). To test this CYP1A1 wildtype (WT) and knockout (KO) mice were exposed to dietary TCDD for 35 d (150 ng/kg/d). Mean arterial pres-
sure (MAP) was monitored by radiotelemetry prior to and during exposure. CYP1A1 was measured in kidney, heart, aorta, and mesenteric arteries and luci-
genin luminescence was measured in the heart, kidney, and aorta as an index of su-
peroxide production. We found that TCDD induced CYP1A1 and lucigenin fluorescence in all tissues of WT mice, but not in KOs. In addition, TCDD steadily increased MAP after 15 d of exposure in CYP1A1 WT mice, reaching hyper-
tension at +20 mm Hg, but did not alter MAP in KO mice. TCDD also in-
duced endothelial dysfunction in CYP1A1 WT mice, which was normalized by a superoxide dismutase mimetic, but had no effect in KO mice. These data demon-
strate that TCDD-induced reactive oxygen species, endothelial dysfunction, and hypertension require CYP1A1 and suggest that production of reactive oxygen species may play a role. Supported by R01 HL078914.

2059 TCDD TREATMENT INDUCES HBD-3 EXPRESSION IN HUMAN KERATINOCYTES IN VITRO AND IN ORGAN CULTURE OF HUMAN SKIN BIOPSY.

J. Allen-Hoffman1, 2, N. De Abrew2 and J. Loertscher1. 1Pathology and Laboratory Medicine, University of Wisconsin - Madison, Madison, WI and 2Molecular and Environmental Toxicology Center, University of Wisconsin - Madison, Madison, WI.

TCDD, a ubiquitous and potent environmental contaminant, has been shown to cause the human skin pathology chloracne. Unlike skin conditions such as acne vulgaris, chloracne patients exhibit reduced microbial growth in affected areas of skin. Using an in vitro model, we examined the antimicrobial properties of human skin following continuous TCDD treatment. TCDD treatment increased the ex-
pression of the β-defensin class of the antimicrobial host defense peptides. Human β 
defensin-1 (hBD-1) and human β defensin-3 (hBD-3) mRNA were found to be upregulated by 12 days of treatment. The induction of hBD-3 mRNA and the concomitant increase in hBD-3 protein was confirmed in adult human skin biopsies treated with TCDD for 18 days. In monolayer culture, exposure of human keratinocytes to TCDD resulted in increased hBD-3 mRNA and protein levels. These results demonstrate that keratinocytes in the absence of mesenchymal cell types, such as the fibroblasts resident to the dermal compartment, are responsible for the TCDD-induced HDP effects. In additional studies, treatment of in vitro skin models with TCDD congeners demonstrated a parallel structure-activity rela-
tionship between the affinity of these chemicals to the aryl hydrocarbon receptor (AhR) and both increased hBD-3 mRNA levels and hyperkeratinization. These re-
results provide indirect evidence for the AhR / AhR Nuclear Translocator (ARNT) pathway in the induction of hBD expression. Cutaneous barrier function of skin tissues, as measured by electrical impedance-based capacitance, was not reduced by 18 days of TCDD-treatment suggesting that the permeability of skin tissue was not affected by treatment. Taken together, these results suggest that TCDD enhances the innate antimicrobial function of human skin through the activation of β-de-
fensins, providing an explanation for the reduced levels of microbial growth fre-
quently seen in patients with the skin condition chloracne.

2060 TCDD INDUCES DERMAL ACCUMULATION OF KERATINOCTYE-DERIVED MATRIX METALLOPROTEINASE-10 IN A THREE DIMENSIONAL MODEL OF HUMAN SKIN.

C. Thomas-Virnig1, N. De Abrew2, C. Rasmussen1, E. Bolterstein2, S. Schlosser2 and J. Allen-Hoffman1, 2. 1Pathology and Laboratory Medicine, University of Wisconsin Madison, Madison, WI and 2Molecular and Environmental Toxicology Center, University of Wisconsin Madison, Madison, WI.

The epidermis of the skin is the first line of defense against the environment. An organotypic model of human skin was used to investigate tissue-specific phenotypes induced by the environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Continuous treatment of an in vitro model of human skin with TCDD resulted in intracellular spaces between keratinocytes of the basal and immediately superbasal layers as well as thinning of the basement membrane, in addition to the previously reported hyperkeratinization. These tissue remodeling events were pre-
ceded temporally by changes in expression of the extracellular matrix degrading en-
zyme, matrix metalloproteinase-10 (MMP-10). MMP-10 mRNA and protein were highly induced following TCDD treatment. qPCR and immunoblot results from TCDD-treated monolayer cultures, as well as indirect immunofluorescence and immunoblot analysis of TCDD-treated skin tissues, showed MMP-10 was specifi-
cally contributed by the epidermal keratinocytes but not the dermal fibroblasts. Keratinocyte-derived MMP-10 protein accumulated over time in the dermal com-
partment of the skin model. TCDD-induced epidermal phenotypes were attenu-
ated by the keratinocyte-specific expression of tissue inhibitor of metalloproteinase-
1, a known inhibitor of MMP-10. These studies suggest that MMP-10 and possibly other MMP-10-activated MMPs are responsible for the phenotypes exhib-
ted in the basement membrane, the basal keratinocyte layer, and the cornified layer of TCDD-treated skin tissues. Our studies reveal a novel mechanism by which the epithelial-stromal microenvironment is altered in a tissue-specific manner thereby inducing structural and functional pathology in the interfollicular epidermis of human skin.
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Several polychlorinated biphenyls (PCB) with three or four

Iowa City, IA.

enantiomeric composition of polychlorinated biphenyls may have human health

toxicokinetic parameters between PCB enantiomers suggest that changes in the

ferences in the toxicokinetic parameters of both enantiomers. The differences in the

ond eluting enantiomers; whereas, the opposite trend was observed for the accumu-

clearance of first eluting enantiomers in blood was faster compared to that of sec-

longer compared to the second eluting enantiomers. The bioavailability normalized

E1-PCB 95, (-)-PCB 132, and (-)-PCB 149 had half-lives that were distinctively

95, 132, 136, 149, 174, and 176 to test the hypothesis that the enantiomeric en-

C57Bl/6 mice after oral administration of a mixture containing racemic PCBs 91,

and enantiospecifically interact with target molecules such as Ryanodine receptors.

Health Institute, Kuopio, Finland and 4National Institute for Public Health and the

and 1B1 suggest that PCBs 180 and 52 do not exert characteristics of AhR agonists.

We hypothesized that exposure to PCBs alters telomerase activity and telomere length. To explore this possibility, we exposed immortal human skin keratinocytes (HaCat) to PCB congers 28, 52, 126, 153 and a synthetic Chicago Air Mixture (CAM) at a concentration of 5μM for eight weeks. Medium and compounds were changed every 3 days. Cells were trypsinized, counted, and re-seeded at low density every sixth day. The remaining cells were used to measure cell viability and cell cycle distribution by flow cytometry and telomerase activity and telomere length by RT-

PCR. None of the treatments were cytotoxic. The population doubling times and cell cycle distributions were similar in all treatment groups except PCB126, where a doubling of cells in S-phase compared to controls was observed. PCBs 28, 52 and CAM produced a significant reduction in telomerase activity from week three to eight. PCB126 and PCB153 significantly reduced telomerase activity from the first week on, reaching an almost 50% reduction of activity compared to control by week eight. In the PCB126- and PCB153-treated groups telomeres began to shorten at week five and lost 50% of their mean length in week eight. In summary, several PCB congeners and the synthetic Chicago Air Mixture reduced telomerase activity and telomere length, indicating that constant exposure to these compounds could lead to premature senescence, genomic instability and cancer. Further studies are needed to explore the mechanisms and consequences of reduced telomerase activity and telomere length. (Supported by NIEHS P42 ES013661, ES 05605, and DOD DAMD17-02-1-0241)

2062 BIOAVAILABILITY NORMALIZED CLEARANCE OF POLYCHLORINATED BIPHENYL ENANTIOIMERS IS ENANTIOSELECTIVE IN FEMALE C57BL/6 MICE.

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Several polychlorinated biphenyls (PCB) with three or four ortho chlorine substitu-

tsents are chiral. These PCB congeners undergo enantiomeric enrichment in vivo and

enantiospecifically interact with target molecules such as Ryanodine receptors. The present study investigates the toxicokinetics of PCB enantiomers in female C57BL/6 mice after oral administration of a mixture containing racemic PCBs 91, 95, 132, 136, 149, 174, and 176 to test the hypothesis that the enantiomeric en-

richment of PCBs is due to differences in the clearance and half-lives of the respec-

tive enantiomers. On the Chiraix-Dex column, an enrichment of the second elut-

ing enantiomers was generally observed; however, only the first eluting enantiomers (-)-PCB 95, (-)-PCB 132, and (-)-PCB 149 had halflives that were distinctive longer compared to the second eluting enantiomers. The bioavailability normalized clearance of first eluting enantiomers in blood was faster compared to that of sec-

ond eluting enantiomers; whereas, the opposite trend was observed for the accumu-

lation factors in adipose tissue. This is consistent with the slower clearance of the first eluting enantiomer. The only exception was PCB 174, which showed no dif-

ferences in the toxicokinetic parameters of both enantiomers. The differences in the

toxicokinetic parameters between PCB enantiomers suggest that changes in the enantiospecific composition of polychlorinated biphenyl may have human health implications because of their enantiomeric toxicity.

2063 LEVELS OF DIOXINS AND POLYBROMINATED

DIPHENYL ETHERS IN HUMAN MILK IN NORTHERN

CHINA AND THE RELATED DIETARY RISK FACTORS.

F. Kyuama1, S. Sun1-2, J. Zhao1, J. Leng1, H. Fukatsu1, D. Liu1 and X. Liu1.

1Center for Community Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan, 2College of Public Health, Hebei Medical University, Shijiazhuang, Hebei, China, 3The Second Hospital, Hebei Medical University, Shijiazhuang, Hebei, China, 4School of Public Health, Peking University, Beijing, Beijing, China and 5S.R.L. Inc., Hashi, Tokyo, Japan. Sponsor: M. Denison.

The concentrations of persistent organic pollutants (POPs), such as dioxins, dioxin-like polychlorinated biphenyls (Co-PCBs) and polychlorinated diphenyl ethers (PBDEs) were detected by high-resolution gas chromatography/high-resolution

tion mass spectrometry in human milk collected from 20 women in Shijiazhuang, Tianjin and Yantai areas in northern China, respectively. Our results show that the highest geometric concentration of total dioxins and total PBDEs were in Tianjin and Yantai, respectively. Based on the results of an in-person interview of mothers using a questionnaire, freshwater fish consumption was found to correlate with total mono-ortho Co-PCBs and sea fish consumption was found to correlate with total non-ortho Co-PCBs of body burdens in these areas. But no correlation was found between concentrations of total PBDEs and total dioxins and consumption of food. Our results indicate that comprehensive monitoring of POPs in the envi-

ronment as well as in food is urgently needed in China to prevent environmental contamination by POPs.

2064 SUPPRESSION OF TELOMERASE ACTIVITY AND

EROSION OF TELOMERES BY PCB CONGENERS AND

MIXTURES: A POSSIBLE NEW MECHANISM OF PCB

CARCINOGENESIS?

S. Phk, J. Jocabus, H. Lehmler, L. Robertson and G. Ludewig. Human Toxicology program, The University of Iowa, Iowa city, IA.

Polychlorinated Biphenyls (PCBs), a group of 209 different congeners, are ubiqui-

tous environmental pollutants. They induce cancer in rodents and are classified as

probable human carcinogens. A hallmark of carcinogenesis is chromosome instabil-

ity and immortality, which are induced by telomere length and telomerase activ-

ity. We hypothesized that exposure to PCBs alters telomerase activity and telomere length. To explore this possibility, we exposed immortal human skin keratinocytes (HaCat) to PCB congers 28, 52, 126, 153 and a synthetic Chicago Air Mixture (CAM) at a concentration of 5μM for eight weeks. Medium and compounds were changed every 3 days. Cells were trypsinized, counted, and re-seeded at low density every sixth day. The remaining cells were used to measure cell viability and cell cycle distribution by flow cytometry and telomerase activity and telomere length by RT-

PCR. None of the treatments were cytotoxic. The population doubling times and cell cycle distributions were similar in all treatment groups except PCB126, where a doubling of cells in S-phase compared to controls was observed. PCBs 28, 52 and CAM produced a significant reduction in telomerase activity from week three to eight. PCB126 and PCB153 significantly reduced telomerase activity from the first week on, reaching an almost 50% reduction of activity compared to control by week eight. In the PCB126- and PCB153-treated groups telomeres began to shorten at week five and lost 50% of their mean length in week eight. In summary, several PCB congeners and the synthetic Chicago Air Mixture reduced telomerase activity and telomere length, indicating that constant exposure to these compounds could lead to premature senescence, genomic instability and cancer. Further studies are needed to explore the mechanisms and consequences of reduced telomerase activity and telomere length. (Supported by NIEHS P42 ES013661, ES 05605, and DOD DAMD17-02-1-0241)

2065 PHOTO-ACTIVATED TITANIUM DIOXIDE

NANOPARTICLES INDUCE TOXICITY THROUGH AN

OXIDATIVE STRESS MECHANISM IN ZEBRAFISH

EMBRYOS.

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Although previous studies report titanium dioxide nanoparticles (TiO2-NPs) as nontoxic, absorption of light energy a the band gap results in separated electrons (e-)

and holes (h+), which form reactive oxygen species (ROS) upon interaction with H2O. Because shorter-wavelength light is absorbed by the NPs, we generated these e-·h+ pairs, which we used to investigate possible toxicological implications of TiO2-NP photochemistry on development. Oxidative conditions, where ROS outnumber their neutralizing antioxidant counterparts, result in ROS indiscriminately damaging cellular macromolecules, leading to cellular death. Therefore, we hypothesized that photo-activated (p-) TiO2-NPs induce oxidative toxicity in zebrafish embryos. Embryos exposed to graded concentrations of TiO2-NPs (75% anate; 25% rutile) under simulated sunlight from 4-120 h post-fertilization had significantly higher mortality and incidence of malformations. In summary, this work extends our understanding of TiO2-NPs as potential environmental contaminants and emphasizes the need for future studies involving TiO2-NPs in the aquatic environment.
genic line Tg(are:eGFP) was created and reported activation of the antioxidant re-
response element only in embryos exposed to p-TiO$_2$NPs. Assessment of DNA dam-
age by immunodetection using 8-OHdG as a marker showed oxidized de-
oxyguanosine in head and yolk sac regions. A transgenic line capable of overcoming
oxidative conditions was established to verify oxidative stress as the cause of the ob-
served toxic effects. Pulse exposures, TEM, and spectroscopy revealed that
TiO$_2$NPs were taken up and physically associated with embryos. Overall, these data
support the idea that p-TiO$_2$NPs lead to oxidative stress and toxicity.

2066 DERMAL ABSORPTION OF ZNO PARTICLES FROM
SUNSCREENS.

B. Gulson $^{1,2}$, M. McCaul$^1$, L. Gómez$^1$, M. Korscht$^2$, P. Casey$^4$ and L. Kinsley$^5$.
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Canberra, ACT, Australia.

Incidence of skin cancer is increasing globally. Skin-care products containing metal
oxide particles can provide UV protection. Recent reviews conclude there is negli-
gible dermal penetration of nanoparticles but concerns remain. We have under-
taken trials with human volunteers using the stable isotope approach to discrimi-
nate Zn absorbed from sunscreen compared with other sources (e.g food). In the
trials particles of ZnO (enriched to 99% in 68Zn) were incorporated into two for-
mulations. Two groups (n=10) of various ages, skin classifications and race, partici-
pated in a study at a Sydney beach in March 2009: one group was treated with a
sunscreen containing nanoparticles of 68ZnO (20nm) and the other group with
larger particles of 68ZnO ≤100nm. Sunscreen was applied to the backs twice daily
for 5 days; subjects experienced a minimum of 1 hour UV exposure in two episodes
following sunscreen application. Blood was sampled twice daily and urine three
times daily. Blood and urine samples were also collected pre- and post-trial. Zn was
purified from blood and urine samples by ion exchange procedures. Changes in the
isotopic abundance of 68Zn in the purified samples, measured by multi-collec-
tor inductively-coupled plasma mass spectrometry, were used to evaluate the der-
mal absorption of zinc from the sunscreens. Blood and urine provide unequivocal
evidence for dermal absorption of Zn in both groups. Excluding outliers the mean
increase in blood is about 0.4% and there was no statistically significant dif-
ference between the groups. Both groups showed significant 68Zn increases in
blood 6 days after completion of the trial. Urine samples show larger increases over
the same time intervals. It may not be possible to confirm whether the Zn is pres-
ent as nanoparticles or soluble Zn ions species because the amounts of 68Zn in the
blood and urine samples are so small. It is not known if levels of 68Zn from sun-
screen would continue to increase or reach equilibrium if the trial were conducted
over a longer term representing life-time use of sunscreens.

2067 ASSESSMENT OF UVB-DAMAGED SKIN JV IVIVO WITH
SUNSCREEN FORMULATIONS CONTAINING TITANIUM AND ZINC NAPARTICLES.

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Monteiro-Riviere$^1$.
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State University, Raleigh, NC and $^2$BASF SE, Ludwigshafen, Germany.

Titanium (Ti) and zinc (Zn) nanoparticles (NP) are commonly added to sunscreens
to protect the skin from ultraviolet B (UVB) radiation. However, data on Ti and Zn
NP disposition in UVB-damaged skin is limited. The dorsum of weanling pigs was
exposed to 110-120mJ/cm$^2$ UVB (2.5 minimal ery-
themal dose) that resulted in moderate erythema (sunburn). Skin was dermatomed
and placed in flow-through diffusion cells for 24h. The UVB-exposed skin and the
non-UVB exposed skin (control) were treated with 4 sunscreen formulations
and placed in flow-through diffusion cells for 24h. The UVB-exposed skin and the
non-UVB exposed skin (control) were treated with 4 sunscreen formulations
(n=6/treatment): 10% coated TiO$_2$ in oil/water; 10% coated TiO$_2$ in water/oil; 5%
coated ZnO in oil/water; and 5% uncoated ZnO in oil/water. TiO$_2$ was
10x50nm size of 140nm (range ca. 60-200nm). Skin was processed for light microscopy (LM), trans-
mition electron microscopy (TEM), and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS). UVB-exposed skin exhibited focal intracellular epider-
mal edema, sunburn cells, dermal inflammation, and microblisters, with residual
sunscreen containing Ti or Zn limited to the stratum corneum (SC). TEM showed
typical UVB damage, with Ti present 17 layers deep in the SC but Zn on the sur-
face of the SC. TOF-SIMS data showed that both the Ti and Zn did penetrate into
the epidermis in both the UVB-exposed and non-UVB skin treated with the sun-
screen formulations. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) de-
tected no Ti or Zn in the perfusate. Perfusion was also concentrated by analytical ul-
tracentrifuge and the sediment analyzed by TEM/EDX, indicating no penetration
of the TiO$_2$ or ZnO NP through the skin. In summary, UVB-damaged skin did not
ehance the penetration of the Ti or Zn NP in the four sunscreen formulations.

2068 IN VITRO PENETRATION STUDIES OF FOUR
SUNSCREEN FORMULATIONS CONTAINING TITANIUM AND ZINC NAPARTICLES IN UVB-
DAMAGED SKIN.

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State University, Raleigh, NC and $^2$BASF SE, Ludwigshafen, Germany.

Sunscreens containing titanium (Ti) and zinc (Zn) nanoparticles (NP) are effective
physical barriers against ultraviolet B (UVB) damage to skin, although little is
known about the disposition of these NP in UVB-damaged skin. In this study, the
backs of weanling pigs were exposed to 110-120mJ/cm$^2$ UVB (2.5 minimal ery-
themal dose) that resulted in moderate erythema (sunburn). Skin was dermatomed
and placed in flow-through diffusion cells for 24h. The UVB-exposed skin and the
non-UVB exposed skin (control) were treated with 4 sunscreen formulations
(n=6/treatment): 10% coated TiO$_2$ in oil/water; 10% coated TiO$_2$ in water/oil; 5%
coated ZnO in oil/water; and 5% uncoated ZnO in oil/water. TiO$_2$ was
10x50nm size of 140nm (range ca. 60-200nm). Skin was processed for light microscopy (LM), trans-
mition electron microscopy (TEM), and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS). UVB-exposed skin exhibited focal intracellular epider-
mal edema, sunburn cells, dermal inflammation, and microblisters, with residual
sunscreen containing Ti or Zn limited to the stratum corneum (SC). TEM showed
typical UVB damage, with Ti present 17 layers deep in the SC but Zn on the sur-
face of the SC. TOF-SIMS data showed that both the Ti and Zn did penetrate into
the epidermis in both the UVB-exposed and non-UVB skin treated with the sun-
screen formulations. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) de-
tected no Ti or Zn in the perfusate. Perfusion was also concentrated by analytical ul-
tracentrifuge and the sediment analyzed by TEM/EDX, indicating no penetration
of the TiO$_2$ or ZnO NP through the skin. In summary, UVB-damaged skin did not
ehance the penetration of the Ti or Zn NP in the four sunscreen formulations.

2069 CERIA ENGINEERED NANOMATERIAL
DISTRIBUTION IN AND CLEARANCE FROM BLOOD: SIZE MATTERS.

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Graham$^3$, R. Sulfara$^3$, S. S. Hards$^5$, D. Butterfield$^4,5$, P. Wu$^6$ and E. A.
Grulke$^1$.
$^1$Pharmaceutical Sciences, University of Kentucky, Lexington, KY, $^2$Toxicology, University of Kentucky, Lexington, KY, $^3$Plant and Soil Science, University of Kentucky, Lexington, KY, $^4$Chemistry, University of Kentucky, Lexington, KY, $^5$Chemistry, University of Kentucky, Lexington, KY, $^6$Center of Membrane Sciences, University of Kentucky, Lexington, KY, $^7$Chemical & Materials Engineering, University of Kentucky, Lexington, KY and $^8$Anatomical Sciences & Neurobiology, University of Louisville, Louisville, KY.

Objectives: To characterize the distribution of ceria engineered nanomaterials
(ENMs) of different sizes in blood and their rate of clearance from the vascular
compartment. Methodologic: Aqueous dispersions of ~5, 15, 30 and 65 nm citrate-sta-
bilized ceria, synthesized and characterized in-house, were intravenously infused
into rats over 1 h. Blood was withdrawn up to 6 times from 10 to 240 min after
completion of the infusion. For the 5 nm ceria, blood was also obtained at 20 h and
30 days. Cerium concentration in whole blood, serum and blood clot was deter-
mined by ICP-MS. Results: Fifteen, 30 and 65 nm ceria ENMs were rapidly re-
moved from circulation, so that 10 min after infusion ± 2% was in blood, com-
pared to ~40% of the 5 nm ceria. From 10 to 240 min, blood concentrations of all
4 ceria sizes decreased with a t1⁄2 of ~ 1 h. The 5 nm ceria showed a second t1⁄2 of
≤ 110 h. The 5 and 15 nm ceria predominantly distributed into serum, the 30 nm
ceria was predominantly associated with blood cells, and the 65 nm ceria was evenly
distributed between the two compartments. Conclusions: Five nm ceria, which was
very resistant to agglomeration and settling in vitro, may not have been as readily
recognized by the reticulo-endothelial system, and was therefore cleared more
slowly, than the larger ENMs. The inverted U-shaped curve showing greatest entry
of 30 nm citrate-coated ceria into blood cells is consistent with a report that 50 nm
spherical gold ENM entered HeLa cells more readily than smaller or larger ones,
showing size matters. Supported by U.S. EPA STAR Grant RD-837772.
Quantum dots (QDs), typically consisting of a cadmium selenide core and zinc sulfide shell, have potential for both diagnostic and therapeutic applications. Examples include tracing transplanted stem cells, cancer imaging, and targeted drug delivery. These materials are expected to be non-toxic due to the biocompatibility of their polyethylene glycol coating. However, coagulation degradation in cellular environments could release metal ions known to adversely affect the developing nervous system and may also physically interfere with cellular processes. In vitro cell models for developmental neurotoxicity often utilize cell lines which extend neurites in response to stimulation with growth factors. Neurite outgrowth can be assessed by multiple measurements including total neurite length, number of neurites, and number of branch points. It is difficult to perform such assessments on statistically relevant populations without automated high-content screening. We are investigating the differentiation of primary neural stem cells isolated from the rat cortex which can be assessed based on their expression of progenitor, neuronal, or glial markers. Cells were seeded onto poly-L-ornithine coated tissue-culture surfaces and exposed to QDs with identical core/shell dimensions and neutral (polyethylene glycol), positive (amine), or negative (carboxyl) surface charges. Fluorescence imaging indicates that QD-exposed QDs associate with neural stem cells to a greater extent than amine or pegylated QDs. Immunocytochemical staining for nestin, a marker for progenitor cells, found that nearly 100% of the cells were in the progenitor state on Day 3. Neuronal differentiation was evaluated by immunocytochemical staining for β-III tubulin. Preliminary results indicate that a short exposure to QDs does not lead to a significant difference in neuronal commitment on Day 7, however, differences have been observed on Day 9 and Day 14. This approach may be an appropriate first-tier screening strategy for nanomaterials that don’t generate an acute toxic response.
More than 50 million women in the United States use hormone replacement therapy (HRT) to relieve menopausal symptoms. However, the public's concern with health risks associated with HRT has led to an increasing interest in the use of alternative botanical-based therapies. While botanical preparations are widely used, limited scientific evidence supports their safe utilization. The collaborative project between the U.S. FDA Office of Women's Health (OWH) and the CDER Informatics and Computational Safety Analysis Staff is aimed at investigating potential hepatic adverse effects that may arise from the use of black cohosh, red clover, hops, and chasteberry. A chemical database describing 577 chemical components of the four botanicals was created from the Natural Products Alert (NAPRALERT) database and other publicly available databases. This computational safety study used a battery of computer-aided tools including advanced structure-activity relationship analysis, systems biology pathway analysis, and chemoinformatic data mining. A systematic assessment of the potential hepatotoxicity of the 577 chemicals we identified in these botanicals is presented, and our assessment will be reviewed by the FDA OWH, Center for Drug Evaluation and Research's FDA Botanical Drug team, and Center for Food Safety and Applied Nutrition's Division of Dietary Supplement Programs. We anticipate that the results of this research will help provide regulatory decision support by prioritizing chemicals for testing and will add to the body of knowledge on these botanicals which are widely used by American women.

**DATA- AND SIMULATION-DRIVEN SYSTEMS FOR PREDICTIVE TOXICOLOGY.**


Toxicogenomics has delivered predictive transcriptomic signatures for renal tubular injury, non-genotoxic carcinogenicity and numerous other pathological phenotypes. The quality and performance of transcriptomic signatures are proportional to the size of the training data set. Larger training data give less overestimation of performance in data-split-validation simulations, and therefore closer estimates to actual forward validation results. But transcriptomic models are best-suited to predict pathology for the species they are evaluated in. Better ways of translating toxic mechanisms in animal models to human biology is key to predictive toxicology. We have begun the development of a top-down, physiological model of mechanisms of liver injury in human, mouse and rat built in collaboration with the Hamner Institute and the FDA. The initial mechanisms modeled include hepatocellular necrosis, apoptosis, cholestasis, steatosis and mitochondrial dysfunction. The use of a mechanistic dynamic model of mechanisms of liver toxicity enables simulation of effects of drugs on liver physiology, expressing the quantitative differences between species, and predictive testing of new drug candidates.

**A NOVEL NON-PARAMETRIC STATISTICAL ALGORITHM IN GENE EXPRESSION ANALYSIS HELPS DIFFERENTIATE PREGNANE X RECEPTOR-DEPENDENT AND INDEPENDENT MECHANISMS OF TOXICITY.**


Genome-wide gene expression profiling has become an increasingly popular method for assessing potential liabilities as well as for elucidating mechanisms of toxicity of drug candidates. Analysis of microarray data is often challenging due to lack of a statistical model that is amenable to biological variation in a small number of samples. Here we present a non-parametric algorithm that requires minimal assumptions about the data distribution. Our method for determining differential expression consists of two steps: i) We apply a nominal threshold on fold change and platform p-value to designate whether a gene is differentially expressed in each treated and control sample relative to the control pool, and ii) Statistical significance is determined by rank-ordering genes based on the number of samples satisfying criterion i between the treated group and control group. The method captures group effect without being too sensitive to anomalies as it allows tolerance for potential non-responders in the treatment group and outliers in the control group. Performance and results of this method were compared with the EBarrays algorithm. These two methods were applied to investigate hepatic transcriptional responses of wild-type (WT) and pregnant X receptor (PXR)-knockout mice after 96 h exposure to an inhibitor of beta-secretase, CMP013. Our results showed that CMP013 led to transcriptional changes in hallmark PXR-regulated genes. The cascade of gene expression changes induced by CMP013 helped explain the dramatic effects on liver including hepatomegaly that were observed in WT animals. Comparison of concordant expression changes between WT and PXR-knockout mice also suggested a PXR-independence association between CMP013 and mitochondrial metabolic perturbations.
2079 A VIRTUAL RAT LIVER TO PREDICT SUSCEPTIBILITY TO DILI IN METABOLIC SYNDROME.
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Metabolic disorders, such as type 2 diabetes and atherosclerosis are a cluster of different conditions caused and mediated by complex multi-molecular interactions. The existence of metabolic syndrome is reported to predispose the liver to DILI, e.g. acetaminophen toxicity, halothane toxicity, etc. Because of the central role of liver in the pathogenesis of metabolic syndrome, we developed a dynamic systems model of integrated carbohydrate, glutathione and fat metabolism in rat liver to study the effect of metabolic syndrome on DILI. The model contains enzymes and transporters whose dynamics are captured quantitatively by ordinary differential equations and predicts cellular metabolite concentrations and flux distributions of cellular processes comparable with experimental observations. We have modified our existing virtual rat liver model with altered signalling and flux distributions of processes like de novo lipogenesis, triglyceride synthesis, phospholipid synthesis, β-oxidation of fatty acids, glucose and fructose metabolism, to create the altered liver observed in metabolic syndrome. We then computationally perturbed various processes to varying degrees in the normal and metabolic syndrome conditioned liver and observed a set of perturbations that seemed to make the liver more susceptible to certain forms of DILI. This generated a set of hypotheses for increased susceptibility to DILI under the influence of metabolic syndrome. We predicted the idiosyncratic toxicity observed in thiothixene zone induced liver injury and demonstrate the key molecular mechanisms responsible. Thus, our approach allows us to identify susceptible patient groups in metabolic syndrome along with drug targets and drug candidates that have increased risk for DILI.

2080 SIMULATING MICROSODIMETRY OF ENVIRONMENTAL CHEMICALS IN EPA'S VIRTUAL LIVER.

U.S. EPA Virtual Liver (v-Liver™) is a cellular systems model of hepatic tissues aimed at predicting chemical-induced adverse effects through agent-based modeling. A primary objective of the project is to extrapolate in vitro data to in vivo outcomes. Agent-based approaches to tissue modeling assume that each constituent cell is an independent system reacting to the microenvironment. For this reason, quantitatively estimating the local nutrient and xenobiotic levels for cells is a prerequisite for modeling the dynamics of cellular responses across micronanometric structures. We model a spatially-extended hepatic lobule connected to a physiologically-based pharmacokinetic (PBPK) model in order to link whole-body exposure through diet or inhalation with cell-scale chemical exposure. We demonstrate that this approach is both efficient for simulating long-term exposure, and flexible, allowing development of detailed models of cellular dynamics. We find that for a simulated compound with minimal metabolism that the average concentration across the simulated lobule is very similar to the predictions for a homogenous, or well-mixed, liver compartment. As the rate of metabolism is increased, however, fluctuations in local concentration arise indicating that cellular function, but not geometry alone, can generate spatial inhomogeneity. When evaluated with a simple threshold model for hepatotoxicity we observe that results can potentially be different in a spatially-extended lobule than would be predicted for a homogenous lobule. By relating environmental exposure to cell-driven fluctuations across a hepatic lobule we have produced a useful tool for virtual tissues in general. EPA reviewed this work but it does not necessarily reflect official Agency policy.

2081 MODELING NUCLEAR RECEPTOR-MEDIATED ACTIVITY AND HEPATOTOXICITY WITH BOOLEAN NETWORKS.

Predicting the human health risk of chronic exposure to environmental contami-"nants remains an open problem. Chronic exposure to a wide array of chemicals – e.g., xenobiotics, perfluorinated chemicals and phthalates – has been associated with a range of hepatic lesions in rodents that can progress to cancer, but extrapolating these effects to humans remains challenging. The U.S. EPA Virtual Liver (v-Liver™) project applies in silico methods to gain insight into in vitro assay results and the pathways perturbed by chemicals, their relationship to adverse effects, and the degree of conservation between rodents and humans. As a proof of concept, we focus on 20 environmental chemicals in the ToxCast™ project that activate nuclear receptors (NR) and are implicated in rodent liver cancer. We developed a model of NR-mediated molecular interactions using curated information from the v-Liver Knowledgebase (KB). The model captures the putative signaling, gene expression and enzymatic interactions mediated by CAR, PXR, PPAR-α, LXR and FXR in human hepatocytes. A Boolean Network formalism was used to simulate pathway perturbations and cross-talk due to the activation of NRs by environmental chemicals. We investigated the dynamics of the system using human in vitro data on NR activation from ToxCast. The 20 chemicals perturbed the system by differentially activating NRs, resulting in variable changes to downstream gene expression and protein activation. The NR-mediated network is being further developed to investigate signaling cascades that are potentially responsible for the altered hepatocellular phenotypes observed in acute tissue lesions.

2082 OVERVIEW OF CURRENT DEVELOPMENT AND REGULATORY EXPERIENCE OLIGONUCLEOTIDE-BASED THERAPEUTICS: CASE STUDIES FOR DIFFERENT CLASSES OF OLIGONUCLEOTIDES.
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The field of oligonucleotide (ON) therapeutics is expanding rapidly, with applications to a broad array of molecular targets and disease indications. In general, various classes of ONs are categorized by their mechanism of action. Historically, the most familiar subclass is comprised of single-stranded DNA antisense ON, where hybridization to specific mRNA sequences inhibits expression of targeted proteins. Antisense ONs have been intensively investigated for nearly two decades, with one approved product and numerous others undergoing clinical development, several of which have recently been reported to exhibit compelling clinical pharmacology. Another type of application is the aptamer subclass. These molecules are identified through an elaborate screening process that selects for high affinity binding of a target protein. Thus far, one ON aptamer has been approved, and several other are undergoing clinical evaluation. As more is learned about RNA biology, the field has expanded to include therapeutic ONs that work through novel molecular mechanisms. An example is the emerging subclass of small interfering RNAs (siRNAs), which are double-stranded RNA molecules that act through RNA interference (RNAi). These siRNAs also inhibit expression of proteins via targeted hybridization to specific mRNA sequences. The pharmacologic potency of these molecules has been impressive in nonclinical investigations, and several have entered the clinic. On the horizon are several new applications of ONs involving modulation of gene expression, and the one that has garnering most attention is the microRNA subclass. The expansion of the potential therapeutic utility of ONs is driven by a boom in the appreciation of the native role that RNA plays in regulation of the production of proteins through endogenous antisense, RNAi or micro-RNA interactions. This session will provide an overview of the regulatory perspective on development of ON-based therapeutics and will provide several examples of development programs that represent the various subclasses of ONs.

2083 SEEKING FUNDING FOR UNDERGRADUATE RESEARCH.
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Most undergraduate professors are adept at finding teaching and learning resources for their classrooms and students. It is often more difficult, however, for them to readily know where to go for research funding. Additionally, many undergraduate faculty may find the information posted on National Institutes of Health (NIH) or National Science Foundation (NSF) Web sites intimidating and difficult to navigate if the faculty are not used to the language of granting bodies or institutions. Available grants could be in the form of classroom and teaching enhancement, professional development, or research opportunities for faculty and students. Both the NIH and the NSF have grants specifically tailored to the needs of undergraduate students and faculty. This session will provide undergraduate faculty with the opportunity to hear presentations from representatives from both federal programs, and to ask questions of each. The goal is to link toxicity faculty and undergraduate teaching institutions with appropriate contacts at the NIH and NSF, as well as encourage them to apply for funding. Such opportunities will directly benefit the faculty and students, thus strengthening the future applicants for toxicology programs around the nation.
et al., 2005). However, this effect has not been substantiated in the literature using selective small molecule 11β-HSD1 inhibitors in in vivo studies. A series of benzamide 11β-HSD1 inhibitors (rat 11β-HSD1 Ki = 100-200nM; rat 11β-HSD2 IC50=10,000nM) were tested in 14-day rat oral gavage toxicity studies. Key results included decreased prostate, seminal vesicle and/or epididymis weights and morphological evidence of atrophy in those androgen sensitive organs. To further explore whether testosterone was playing a role in these male reproductive organ changes, a selective benzamide 11β-HSD1 inhibitor was tested using a 14-day male rat surgical castration model (sham controlled +/- testosterone propionate). Treatment with an 11β-HSD1 inhibitor decreased accessory sex gland weight and decreased organ weights and morphological changes were inhibited with supplemental testosterone propionate. 11β-HSD1 inhibitor treatment resulted in an increase in the expression of genes associated with testosterone degradation (1β,25 – HSDDII) in the testes. Supplementation with testosterone propionate reversed the up regulation of 17β-HSD2 and also decreased STAR, Cyp17a1, Cyp17a1 which are responsible for androgen synthesis in the testes. These results suggest that these 11β-HSD1 inhibitors caused reproductive toxicity via a testosterone dependent pathway, most likely through the induction of enzymes responsible for testosterone degradation.

Nuclear receptor agonists such as phenobarbital (PB), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), and 3-methylcholanthrene (3-MC) decrease circulating thyroid hormone (T3). T3 degradation occurs due to the induction of hepatic metabolizing enzymes resulting in an enhanced catabolism of T3. Using primary rat hepatocytes, we compared the effects of constitutive androstane receptor (CAR) and aryl hydrocarbon receptor (AhR) agonists on T3 catabolism. Sprague-Dawley rats were treated with one of the following: CAR agonist, PB (100 or 1000uM) or PCB 153 (3 or 30uM) or AhR agonist, 3-MC (1 or 5uM). Control and treated cells were exposed to a final concentration of 0.1% dimethylsulfoxide (DMSO). After 72 hours, media were removed and replaced with 0.05uM [125I]-T3 (rat median serum concentration). After 24 hours, media and cell lysates were collected for analysis. T3 and its metabolites were separated using an Ultra Performance Liquid Chromatography (UPLC) from which fractions were collected and quantified on a gamma counter. The predominant metabolite found in the media of control and treated hepatocytes was T2-glucuronide (T2G). Following a 24 hour incubation of 0.05 to 1000uM (10²)-T3 with control hepatocytes, Vmax and Km for T3 formation was 8.0 pmol/mg protein/min and 37.6uM, respectively. PB did not significantly increase the formation of T3G in comparison to control hepatocytes. However, initial studies with 3-MC (1 and 5uM) and PCB 153 (3 and 30uM) show significant increases in T3G in media (2.7-3.4-, 1.8- and 3.7-fold, respectively). These results suggest that unlike PB, pre-treatment with 3-MC or PCB 153 increases the catabolism of T3 in rat hepatocytes. These observations also demonstrate the utility of primary rat hepatocytes for screening thyroid hormone disruptors. (This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA or NIH policy.)

We studied the possible effects of in utero and lactation exposure to bisphenol A (BPA) on learning behavior and brain histology in offspring of mice. Pregnant C57BL/6j mice in testing groups were exposed to either 0, 0.33, 3.3 or 33 ppm of BPA (-0, 0.05, 0.5 or 5 mg/kg/day, respectively) in diet from the 6th day of gestation to the 22nd day of lactation. A food motivated serial learning test was conducted to examine memory and learning function after BPA exposure in the male offspring at 9 weeks of age for 10 days consecutively. On the last day of the serial learning task, animals were euthanized with overdose of anesthetic. Specimens of the brains were examined for histological modification. The c-fos positive cells in the selected areas, forebrain, hippocampus, striatum, accumbens, of the brains of BPA-exposed groups were counted and compared with animals from those of the control group. In the serial learning test, the numbers of animals which mastered the learning task in BPA-exposed groups were fewer than those in the control group. However, the memory and learning function at the last session of the serial learning test did not differ among groups. In the brain histological examination, the numbers of c-fos positive cells in all brain areas did not show differences between exposed and control animals. The results of this study indicated that there were neither clear toxic effects on the serial learning test nor brain histology in male offspring following in utero and lactational exposure to BPA were not clearly observed.

Previously, mice were found to show a trend for increased body weights and reduced brain weights. However, these changes did not achieve statistical significance. Effects Research Group, National Institute of Occupational Safety and Health, Japan, Kawasaki, Kanagawa Prefecture, Japan and 2Division of Health Effects Research Group, National Institute of Occupational Safety and Health, Japan, Kawasaki, Kanagawa Prefecture, Japan. Sponsor: M. Fuma.

Recent work has shown that a single oral administration of atrazine (ATR), a chlorotriazine herbicide, induces dose-dependent increases in plasma adrenocorticotropic hormone (ACTH) and serum corticosterone (CORT), with a NOEL of 5mg/kg. The mechanism for these effects is unknown. To test whether administration of ATR causes hypothalamic-pituitary-adrenal (HPA) axis activation through the production of a generalized stress response resulting from gastrointestinal distress, we conducted both conditioned taste aversion (CTA) and pica behavior experiments. CTA is a classical conditioning paradigm that uses single trial learning of an association between consumption of a novel food (i.e. sucrose) and the development of an altered internal state, not necessarily gastrointestinal distress per se. Alternatively, pica behavior (consumption of a non-nutritive substance, i.e. kaolin) is displayed by animals given nausea-inducing chemicals that do not possess an emetic (vomiting) reflex. Adult male Wistar rats were given a single oral dose of ATR (0, 5, 25, 50, 100, or 200mg/kg), the primary ATR metabolite desethyl-desisopropyl atrazine (DACT; 135mg/kg), or lithium chloride (LiCl, i.p.; 12mg/kg). Results from the CTA experiment demonstrated a clear dose-response for ATR, with a NOEL of 5mg/kg. Animals dosed with DACT or LiCl developed aversions comparable to the highest dose of ATR. The pica experiment showed that lower doses (5-50mg/kg) of ATR had no effect, as measured 6 and 24 hours post-dosing, nor did DACT. However, the highest dose of ATR (200mg/kg) and LiCl did induce pica behavior at both time points. These data demonstrate that increases in ACTH and CORT secretion following administration of ATR occur at doses that are without effect on the display of pica behavior, indicating that the HPA-axis activation caused by ATR is not the result of gastrointestinal distress. This abstract does not necessarily reflect U.S. EPA policy.
2088 EVALUATING THE INVOLVEMENT OF GLUCOCORTICOID FEEDBACK ON THE REPRODUCTIVE EFFECTS OF ENVIRONMENTAL CHEMICALS.

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Acute and chronic stressors activate the hypothalamic-pituitary-adrenal (HPA) axis and are known to suppress reproductive function through central negative feedback of the gonadal axis by glucocorticoids. Recently, several environmental chemicals known to attenuate or suppress the lutetizing hormone (LH) surge necessary for ovulation have also been shown to activate the HPA axis. We hypothesize that the attenuation of the LH surge by some of these HPA axis-activating compounds results from the central negative feedback effects of glucocorticoids. To test this hypothesis, the current study exposed female, regularly cycling, Long-Evans hooded rats to a single oral dose of 150 mg/kg sodium (MS) or 0.1% methyl cellulose vehicle during the proestrous critical period (1100-1300 hours). This dose of MS stimulates a dramatic increase in adrenocorticotropic and corticosterone (CORT) within fifteen minutes of oral exposure and blocks the LH surge in proestrous rats. Acute CORT production to MS was inhibited by subcutaneous injection of the 11β-hydroxylase inhibitor Metyrapone (MET) 40 minutes prior to oral exposure but unaffected by polyethylene (PEG) vehicle. Serum concentrations of LH during the surge (1800 hours) were suppressed in both PEG/MS- and MET/MS-treated rats compared to methyl cellulose-treated rats. These results indicate that suppression of MS-induced CORT secretion by MET does not rescue the LH surge. We conclude that glucocorticoid feedback following MS exposure is not the mode of action by which this compound suppresses the LH surge. Future studies will use this experimental design to determine the role of corticosteroid feedback on the reproductive effects of other environmental chemicals. This abstract does not necessarily reflect EPA policy.

2089 DIETHYLHEXYL PHTHALATE (DEHP) EFFECTS ON TESTOSTERONE PRODUCTION IN BLTK1 MOUSE LEYDIG CELLS.

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DEHP is a widely used plasticizer found in a variety of products. In vitro exposure studies have reported that high doses of DEHP are associated with male reproductive tract abnormalities in rats. To further investigate the effects of DEHP and its primary metabolite, monoethylhexyl phthalate (MEHP), we used BLTK1 cells, a subclone of a murine Leydig cell line (BLT-1 cells, clone K1) isolated from a testicular tumor obtained from a transgenic mouse expressing the mouse inhibin alpha subunit promoter/simian virus T-antigen fusion gene. BLTK1 cells exhibit limited basal steroidogenesis, but express Star, Cyp11a1, Cyp17a1, Hsd3b1 and Hsd17b3 as confirmed by RT-PCR and/or western analysis. In addition, 3 ng/mL recombinant human chorionic gonadotropin (hCG) induced progesterone (P) -90-fold and testosterone (T) -50-fold compared to basal levels, as well as upregulated Cyp17a1 and Hsd17b3 mRNA levels. In 24 hr dose-response studies, 0, 3, 10, 30, and 100, and 300 μM DEHP had no effect on P or T levels. In contrast, MEHP induced P -90-fold and T -15-fold compared to non-stimulated basal levels in a dose-dependent manner. In time course studies (1, 2, 4, 8, 12, 24, and 48 hrs) maximum P and T levels were achieved at 4 hrs for 3 ng/mL rhCG compared to 18-24 hrs for 1 ng/mL rhCG. The precipitated proteins are removed and the formed T4-glucuronide is analyzed with protein concentrations between 0.1 and 1.5 mg/mL; the induction of liver enzymes by Aroclor 1254 resulted in factor 1.6 higher T4-specific UDP-glucuronosyltransferase activity which is used for quantification of the receptor activation by luminescence measurement. The assay is used to detect agonistic effects of substances on the human estrogen receptor-alpha. In our laboratory we have optimized and validated yeast-based assays to detect agonistic and antagonistic effects of substances on the human estrogen receptor-a (YES) and androgen receptor (YAS). Similar to the HEla assay, the gene coding for the human estrogen receptor-a has been integrated into the genome of a yeast strain. Additionally, the yeast contains a plasmid carrying the lac Z gene, which is receptor-dependently expressed and serves as a reporter gene. The lacZ activity is used to quantify the activation of the human estrogen receptor-alpha. The YES was validated with approximately 100 test compounds and the assay is currently used in screening more than 50 substances per year. We have compared the performances of the HEla-cell and the yeast-based assays. Both assays perform with similarly, but we prefer the yeast-based assays for their significantly greater robustness and the option to study agonistic effects of substances on the estrogen receptor-a (YES) and androgen receptor (YAS). In rats, the higher plasma turnover of T3 and T4 is associated with an increase of TSH leading to thyroid proliferation and eventually in thyroid tumors. This represents an indirect mechanism of tumorigenesis. And rats - in contrast to humans - are highly sensitive to this mechanism. Usually the mechanism of thyroid hormone disruption in rats is studied by perchorlate discharge assays (for direct effects), T4-glucuronide kinetics or -glucuronidation assays using radioactive [1\textsuperscript{25}]T4 (for the indirect effect described above). We describe a method to determine the induction of T4-specific UDPGT ex vivo using HPLC-MS: Rats are treated for up to 2 weeks with the test compound; untreated rats serve as controls. Liver microsomes are prepared and solubilized with surfactant (the enzyme is located on the lumen side of microsomes). The enzyme preparation is then incubated with T4 and the cofactor UDP-Glucuronic acid. The incubation is stopped by adding ice-cold acidic methanol. The precipitated proteins are removed and the formed T4-glucuronide is analyzed in the supernatant by LC-MS analysis. The method was tested for several concentrations of microsomal protein and for microsomes of untreated rats and rats treated with the inducer Aroclor 1254. The T4 glucuronidation correlated linearly with protein concentrations between 0.1 and 1.5 mg/mL; the induction of liver enzymes by Aroclor 1254 resulted in factor 1.6 higher T4-specific UDP-Glucuronosyltransferase activities. Taken together, the determination of T4-specific-UDP-GT induction by LC-MS analysis of the glucuronide offers a non-radioactive method to detect specific hepatic enzyme induction which contributes to thyroid proliferation and eventually tumor formation in rats.
2093 CADMIUM-INDUCED PANCREATIC ISLET β-CELLS DYSFUNCTION AND CELL DEATH: THROUGH ROS MEDIATED MAPK-MITOCNDRIAL DEPENDENT APOPTOSIS PATHWAY.

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Cadmium is a well-known environmental pollutant. Cadmium has been demonstrated that accumulates in the pancreas and exerts diabetogenic effects in animals. However, the precise effects and mechanisms of cadmium on the pancreatic β-cells remain unclear. In this study, we attempted to investigate the molecular mechanisms of cadmium on induced the dysfunction and cell death of pancreatic β-cell line RIN-m5F. After CdCl₂ (1-10 μM) treatment for 24 h, cell viability was obviously reduced and the insulin secretion could also be suppressed in a dose-dependent manner. 2',7'-Dichlorofluorescien fluorescence and lipid peroxidation (LPO) levels as an indicator of oxidative stress formation after exposure of cell to CdCl₂ remain unclear. In this study, we attempted to investigate the molecular mechanisms of cadmium on induced cell apoptosis, including activation of JNK, p38, and β-cell death. Meanwhile, treatment of HIT-T15 cells with CdCl₂ resulted in decreased cell viability and cell death. These results indicate that CdCl₂-induced oxidative stress causes the pancreatic β-cell dysfunction and cell death through ROS mediated mitochondrial-dependent apoptosis pathway. Our data provide evidence that cadmium may be an important environmental risk factor for diabetes progressing.

2095 MERCURY CHLORIDE-INDUCED PANCREATIC β-CELL DEATH: INVOKE OF APOPTOSIS AND NECROSIS.

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Mercury is a well-known highly toxic metal; exposure to mercury induces reactive oxygen species (ROS) production and causes severe damage in mammal including pancreatic β-cells, which are vulnerable to oxidative stress. However, the toxic characterization and action mechanisms of inorganic mercury in β-cells are still unclear. In this study, we explore the cytotoxic effects of HgCl₂ in pancreatic β-cell-dereived HIT-T15 cells. HgCl₂-induced concentration-dependently (range from 2 to 20 μM) decreased the number of viable cells after treatment for 24 h. Fluorescent levels of DCFH-DA as an indicator for ROS formation, significantly increased after exposure of HIT-T15 cells to HgCl₂. Pre-treatment with antioxidant N-acetylcysteine reversed HgCl₂-triggered ROS generation. Pre-treatment of HIT-T15 cells and primary isolated mouse pancreatic islets with 0.5 μM NAC protected HgCl₂-inuced insulin secretion dysfunction. Moreover, HgCl₂ displayed several features of mitochondrial-dependent apoptotic signaling pathway including an increase of annexin-V+/PI- cells, disruption of the mitochondrial membrane potential, cytochrome c release, and activation of poly (ADP-ribose) polymerase (PARP) degradation and caspase 3 protease. Meanwhile, treatment of HIT-T15 cells with HgCl₂ was also resulted cell necrosis which indicated by increasing of propidium iodide binding (annexin-V+ and PI+ cells), bright orange-red fluorescence population (by dual staining with fluorescence probes of acridine orange and ethidium bromide), and depletion of intracellular ATP levels. Altogether, these results suggest that HgCl₂-induced oxidative stress and caused pancreatic β-cell dysfunction and cytotoxicity is a mixture of both apoptosis and necrosis.
this approach, a chemical can be considered as an endocrine disrupter when a posi-
tive outcome in endocrine sensitive endpoints in an apical (or relevant non-apical in vivo) study is conclusively supported by mechanistic data i.e. the sequence of the biochemical/cellular/organ events that underlies the adverse effect has been described and understood. Once a chemical has been identified as an endocrine disrupter a po-
tency assessment should be performed based on specificity, human relevance, dose level, exposure duration, nature/severity of adverse effects and number of species af-
ected. These elements should be considered collectively in a weight of evidence ap-
proach; together with data on human exposure, the risk that a chemical may pose to human health can then be fully evaluated.

Flavonoids are abundant in plant food and commonly recognized for their estrogenic effects. Many of the flavonoids are known to interact with cytochrome P450 enzymes, including enzymes responsible for steroidogenesis. Genistein (GEN), daidzein (DAI) and apigenin (API) are three flavonoids known to inhibit corticosteroid secretion in vitro and GEN decreases corticosterone and testosterone levels in rats. The human adrenocortical cell line H295R expresses all enzymes required for se-
cretion of adrenal and sex hormones. Herein, we have investigated the effect of the three flavonoids and their ternary mixture on aldosterone (A), cortisol (C), testos-
terone (T) and estradiol (E) secretion. H295R cells were treated with GEN, DAI and API for 24 hours at non-cytotoxic concentrations (0-10 μM), individually and as mixture. Hormone levels in cell culture medium were analysed by ELISA. The flavonoids caused a dose-dependent inhibition of A, C and T, with stronger effects exerted by GEN and DAI than by API. The mixture also affected A, C and T secre-
tion in a dose-dependent way, which followed either of the two prediction models concentration addition (CA) and independent action (IA). GEN and DAI, how-
ever, showed no effect on E secretion, while API inhibited secretion at the highest concentration. Following mixture treatment, E secretion was inhibited at a lower concentration of API. The results indicate that single flavonoid compounds, at no observed effect levels, may add up to an effect on hormone secretion when com-
bined in a mixture. Since these flavonoids are abundant in food, mixture exposure is highly relevant. We conclude that GEN, DAI and API affect steroid secretion fol-
lowing single and mixture treatment and that CA and IA can predict the mixture effect.

The consequences of perchlorate disruption of the HPT axis by blocking thyroidal uptake of iodide are considered to be dependent upon iodide in the diet. The goal of this research was to compare perchlorate-induced disruption of the HPT axis in the iodine deficient (ID) and sufficient (IS) adult Sprague-Dawley rat. One group of rats (n=40) was placed on IS chow (193 ng/g) and another (n=40) on ID chow (16.2 ng/g) for either 54 or 68 days. On Day 53 one-half of the rats from each group were started on drinking water with a high dose of perchlorate (10 mg/kg/day). The serum thyroid hormone levels in the ID rats were 83% lower than the IS rats. After 54 days of ID diet, serum levels of TT4 were slightly decreased, while thyroid gland rT3, T3 and T4 levels were decreased 57 to 74% and thyroid weight increased 44%. After 68 days of ID diet, serum TT4 levels dropped 53% while TT3 (+9%) and TSH (+300%) levels increased. Thyroid gland hormones remained de-
creased by 67% to 83%. One day of perchlorate exposure did not significantly alter the existing condition of the HPT axis for the IS or ID groups; however, 14 days of perchlorate exposure disturbed the HPT axis of both groups. For the ID rats, serum TT4 was not detectable, TT3 levels were reduced by 80% and TSH levels increased 270%. rT3, T3 and T4 in the thyroid glands were not detected in a few samples at any level. IS perchlorate-treated rat serum TT4 (40%) and TT3 (24%) levels were reduced and serum TSH levels increased 276%. Thyroid hormone levels in the thyroid gland were reduced 84 to 96%. (Support: U.S. EPA STAR Cooperative Agreement R823134. This work does not necessarily reflect EPA policy.)

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia caused by defective insulin secretion, resistance to insulin action, or a combination of both. Treating hyperglycemia with drugs that block intestinal glucose uptake and renal glucose reabsorption via the sodium-glucose transporters (SGLT1, SGLT2) represents a novel approach to diabetes treatment. Calculiria and increased trabecu-
lar bone was found in toxicity studies with the mixed SGLT1/2 inhibitor SAR7226 and the low-absorbable and selective SGLT1 inhibitor SAR474832. To investigate whether renaMually excrated calcium was mobilized from bone a specific study in young vs. old rats was conducted with SAR7226 focusing on electrolytes (serum and urine), bone quality parameters (pQCT, DXA, bone biomechanical testing), hormones related to calcium homeostasis, and biomarkers of bone turnover. Treatment with SAR7226 resulted in dose-related, marked glucosuria – the anticipa-
pated pharmacological effect- polyuria, and calciruria. Normal serum calcium was maintained while serum phosphorus was slightly increased. PTH and 1,25 vitamin D3 were markedly suppressed as well as markers of bone turnover. These effects were associated with increases in bone mineral density (BMD). Increases in BMD at the spine were positively associated with increases in bone strength. Effects on bone mass were characterized microscopically by increases in trabecular bone. The SGLT1/2 inhibitor SAR7226 markedly influences calcium and phosphorus home-
ostasis with positive effects on bone mass and strength. Similar effects were ob-
erved with the low-absorbable selective SGLT1 inhibitor SAR474832. From these results it is concluded that calciria during studies with SAR7226 and SAR474832 is not associated with a calcium release from bone. These studies highlight the impor-
tance of considering evaluations of the skeleton in the safety assessment of com-
pounds affecting calcium homeostasis to provide important safety data.
GENISTEIN MODULATION OF BLOOD GLUCOSE LEVELS IN DIABETIC MALE MOUSE MODELS. T. L. Gun1, J. F. Zheng1, D. B. Germolec2 and K. L. White1. 1Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA and 2NIEHS, Research Triangle Park, NC.

Previously, we have reported that oral exposure to the phytoestrogen genistein (GEN), a major isoflavone in most soy products, protected female NOD (non-obese diabetic) mice from developing type 1 diabetes while exacerbating streptozotocin (STZ, a selective β-cell cytotoxin) -induced diabetes in female B6C3F1 mice. The objective of the present study was to determine if oral exposure to GEN (20 mg/kg) modulated blood glucose levels in diabetic male mouse models. In STZ-induced diabetic male B6C3F1 mice, GEN treatment significantly reduced blood glucose levels (320.2 ± 31.5 mg/dL vs. 233.5 ± 29.2 mg/dL) as compared to vehicle controls when mice were fed NTP-2000 diet. In STZ-induced diabetic male db/db C57BL/6 mice, GEN treatment significantly reduced blood glucose levels (534.7 ± 40.3 mg/dL vs. 376.2 ± 45.8 mg/dL) when mice were fed a high fat diet (TD.06414, Harlan). In contrast, GEN exposure significantly increased the incidence of diabetes (blood glucose levels ≥ 250 mg/dL; 13% vs. 56%) and severe diabetes (blood glucose levels ≥ 400 mg/dL; 13% vs. 50%) in male NOD mice two weeks after cyclophamide treatment (200 mg/kg) when mice were fed NTP-2000 diet. The differential effects of GEN on blood glucose levels in male and female mice suggest that the estrogenic properties of this compound may contribute to its modulation of diabetes (Supported in part by NIEHS contract NO1-ES05454).

INTERACTION OF POTENTIAL ENDOCRINE-DISRUPTING CHEMICALS WITH CYP3A4, CYP1A2, P-GLYCOPROTEIN, AND RAT CYP1A1 PROMOTER REGIONS.

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Rational: Two studies, Endometriosis, Natural History, Diagnosis, and Outcomes (ENDO) and Markers Of Risk and ENDO (MORENDO) are underway to determine if there is a link between exposure to endocrine-disrupting chemicals (EDCs) and the expression of endometriosis in women. It has been suggested that the human pregnane X receptor (PXR) could be utilized as a “xenosenor” of EDCs. The arylhydrocarbon (Ah) receptor has long been known to react to environmental contaminants. EDCs encompassed in the ENDO and MORENDO studies have been examined for their ability to activate transcription via PXR and XRE mechanisms at physiological levels. Experimental Procedures: 1)A2DRE (CYP1A2 XRE), DPX2 [CYP3A4 XRE], RXR [rat CYP3A1], and MDR1(C (human P-glycoprotein) cells, obtained from Puracyp Inc., were exposed to increasing concentrations of potential EDCs for 24 hours. Viability was determined by MultiTox (Promega) and luciferase reporter induction was determined by luminescence using BrightGlo (Promega) using a BioT ek Synergy 2 plate reader, EC50s were determined using B-CLEAR® technology. Minor (~20%) effects on E217G cell accumulation, but no significant differences in E217G Clb, were observed. TCS did not alter digoxin Clb in a dose-dependent manner (48% at 30 μM TCS). Thus, TCS may modestly inhibit Mdr1-mediated biliary excretion. Protein content for Cyp3a1, Oatpl1a1, Oatpl1q4, Mrp2, Mdr1p and Mrp4 in SCH was assessed by Western blot. TCS increased Cyp3a1 at all doses; Oatpl1a4 and Mrp2 proteins were slightly increased. These data suggest that while TCS may interact with CAR and PXR to upregulate hepatic cathepsin and decrease circulating T4 concentrations, TCS has minimal effects on hepatic transport of thyroid hormone. Supported by the Colgate-Palmolive Award for Students, NIEHS T32-ES07126, EPA CR833237, and NIH GM41935. This abstract does not necessarily reflect the policy of the U.S. EPA or NIEHS.

PERINATAL EXPOSURE TO PCB52 AND PCB180 INCREASES AROMATASE ACTIVITY IN RAT LIVER, OVARY AND ADRENAL GLAND.

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Polychlorinated biphenyls (PCBs) are still ubiquitous pollutants, despite the ban on their industrial and commercial use. Studies to determine the endocrine disruptive potential of non-dioxin-like (NDL) PCBs have mainly focused on interactions with thyroid hormone, estrogen and androgen receptors. However little is known about their potential interaction with aromatase. (CYP19) a key enzyme in steroidogenesis that converts androgens into estrogens. Two perinatal toxicity studies with Sprague-Dawley rats were conducted with highly purified (>99.9%) PCB52 and PCB180. Dams were exposed to PCB52 and PCB180 by oral gavage in ten and four equal sub-doses, respectively. The total doses were 0-1000 mg for PCB180 and 0-3000 mg for PCB52. Pups were sacrificed at PND 7, 35 and 84. Aromatase activity was determined in microsomes isolated from ovaries, testis, adrenals, and liver. In utero exposure to the highest dose of PCB180 resulted in a significant increase of aromatase activity in the liver of male pups at PND84, although both the basal and induced activity was very low (0.605 and 1.07 pmol/mg protein/h, respectively). In utero exposure to the highest dose PCB52 significantly induced aromatase in female pup adrenals and ovaries at 0.7 and 2.8 pmol/mg protein/h to 10.1 and 7.6 pmol/mg/protein/h at PND84, respectively. In vitro studies with human placental microsomes and human adrenocorticotropinoma cells H295R showed no catalytic effects on aromatase activity by PCB52 nor PCB180, indicating no direct interaction with the aromatase enzyme. These data suggest that especially PCB52 might have endocrine disruptive potential after exposure in utero. Circulating levels of androgens and estrogens are being determined to assess if the changes in aromatase activity observed in vivo can bring about alterations in hormone balance.

TRICLOSAN DISRUPTS THYROXINE CONTRIBUTION OF HEPATIC TRANSPORT TO THE MODE OF ACTION.

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Triclosan ([5-chloro-2-(2,4-dichlorophenoxy)phenol](TCS)] decreased serum thyroxine (T4) and upregulated Phase I and II hepatic metabolism in rats in our previous work. The role of hepatic transporters in the mode of action for TCS-induced hypothyroxinemia has not been evaluated. To test the hypothesis that TCS decreases T4 via activation of the pregnane X and constitutive androstane receptors (PXR and CAR) resulting in upregulation of thyroid hormone transport, both in vivo and in vitro studies were employed. Weanling female Long-Evans rats received TCS (10-000 mg/kg/day) po for 4 days and hepatic transport protein mRNA (Oatpl1a1, Oatpl1q4, Mrp2, Mrp4) was measured by qRT-PCR. No changes in mRNA expression were observed. Rat sandwich-cultured hepatocytes (SCH) were treated with TCS (0-30μM) - non-cytotoxic as determined by lactate dehydrogenase) for 2 or 48hr prior to measuring uptake and biliary clearance (Clb) of estradiol-17β-glucuronide (E217G; Oatpl1q4/Mdr1p probe) and digoxin (Oatpl1q4/Mdr1p probe) using B-CLEAR® technology. Minor (~20%) effects on E217G Clb accumulation, but no significant differences in E217G Clb, were observed. TCS did not alter digoxin cell accumulation, but decreased digoxin Clb in a dose-dependent manner (48% at 30 μM TCS). Thus, TCS may modestly inhibit Mdr1-mediated biliary excretion. Protein content for Cyp3a1, Oatpl1a1, Oatpl1q4, Mrp2, Mdr1p, Mrp3, and Mrp4 in SCH was assessed by Western blot. TCS increased Cyp3a1 at all doses; Oatpl1a4 and Mrp2 proteins were slightly increased. These data suggest that while TCS may interact with CAR and PXR to upregulate hepatic cathepsin and decrease circulating T4 concentrations, TCS has minimal effects on hepatic transport of thyroid hormone. Supported by the Colgate-Palmolive Award for Students, NIEHS T32-ES07126, EPA CR833237, and NIH GM41935. This abstract does not necessarily reflect the policy of the U.S. EPA or NIEHS.

RECRUITMENT OF COREGLUTATORY PROTEINS TO THE ESTROGEN RECEPTOR COMPLEX BY XENOESTROGEN LIGANDS.

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Xenostrogens are a large class of structurally diverse compounds that can interfere with hormonal signaling by binding to the estrogen receptor (ER). Once liganded, ERs recruit coregulatory proteins to form a transcriptional complex that interacts with gene promoter elements. Xenostrogens typically display weaker affinity for the ER relative to 17β-estradiol (E2), which may result in alternate coregulator recruitment, thus modulating the complex. Our goal is to identify and characterize ER complexes in response to a suite of xenostrogens including nonylphenol (NP), bisphenol-A (BPA), genistein, and others. To accomplish this we have utilized a combination of high-throughput screening assays, cell-based experiments and proteomic approaches. Using time-resolved fluorescence resonance energy transfer we observed differential recruitment of coreglulatory proteins to the ER by various ligands. Specifically, E2, NP, and BPA recruited SRC-1 while E2, NP, and genistein recruited SRC-3. To assess ER activation by xenostrogens in MCF-7 cells we employed an ERE reporter gene assay. Results showed that most ligands tested activated the ER despite differential recruitment of the SRCs. These data
imply that recruitment of SRCs to the ER may not be essential for activation at an ERE, but may play a greater role in interactions with other response elements. To determine the functional role of SRC-3 in ER activity, we used siRNA methodology and measured the expression of pS2, a known E2-responsive gene in MCF-7 cells. Knockdown of SRC-3 led to reduction of pS2 by E2, NP and BPA, thus revealing the importance of this coregulator in ER activation. Finally, we have implemented a proteomic approach (DNA column/IP followed by MALDI analysis) for identifying novel ER coregulatory proteins, which has revealed potential candidates upon E2 and NP induction. Overall, our results suggest that xenestroges influence ER transcriptional complex formation and subsequent activity.

2106 GENDER DIFFERENCES IN ESTROGEN RECEPTOR EXPRESSION AND ACTIVATION IN THE LUNG BY XENOESTROGENS.
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Gender differences in the incidence and prevalence of lung diseases such as cancer, COPD and fibrosis may be attributable to hormonally driven signaling mechanisms. There are two types of estrogen receptors (ER): ERα and ERβ. They are ligand-inducible transcription factors that control genomic-mediated estrogen responses and a membrane form (GPR30) which controls non-genomic steroid actions. Few studies have investigated the role of ERs in normal lung function and disease processes. It is of interest to determine if the ERs are expressed in the airway epithelial cells (SAEC) and in lung tissues from patients with idiopathic pulmonary fibrosis (IPF) using qRT-PCR. Furthermore, we are interested in the ability of epithelial cells (SAEC) and in lung tissues from patients with idiopathic pulmonary fibrosis to be mildly estrogenic and our results agree with the liver to BW ratio increases of DE-71, absolute liver weight and liver to BW ratios were significantly higher in all treated groups in a dose related manner. AA rats had a spectrum of treatment related histopathological changes including cytoplasmic alteration such as peripheral hepatocytes; bile duct hyperplasia; and prominent areas of chronic inflammation. DE-71 rats had diffuse hepatic hypertrophy characterized by an increased hepatocellular size adjacent to centrilobular areas which was dose dependent. Serum hormone levels for estradiol, T3, T4, TSH, DHT, FSH, LH, testosterone, and prolactin, were determined. AA high dose rats had a decreased level of T4 and a slightly decreased level of testosterone. Rat testosterone and estradiol had a decreased ratio at the two highest doses and an increased TSH level at 60 mg/kg/day. DE-71 has been shown to be mildly estrogenic and our results agree with the liver to BW ratio increases and hormone responses. AA results confirm its hepatotoxicity. These findings will be compared with those from the second laboratory to determine the variation between laboratories for this assay.

2107 ORGANIZATIONAL VERSUS ACTIVATIONAL EFFECTS OF THE ANTIANDROGEN FLUTAMIDE ON MALE MEDAKA.

Intersex fish have been identified in various contaminated waters around the world. The effects of estrogenic chemicals on male reproductive tract development and function have been welldocumented; however, increasing attention has been placed on the potential involvement of antiandrogens in the reproductive impairments observed in wildlife. The aims of the current study were to characterize the effects of the model antiandrogen flutamide on testis morphology and vitellogenin production in male fish and to compare organizational and activational effects of flutamide. Medaka were treated with a series of flutamide concentrations (0, 0.08, 0.16, 0.31, 0.63, 1.25 mg/L) in either of two dosing paradigms. In the developmental study, Medaka were exposed to flutamide on days 1-14 post hatch and then moved to clean water and reared to sexual maturity. In the adult study, mature males were used for a test of pituitary production of pS2. Testes were removed in small fragments, homogenized, and used for the measurement of vitellogenin (Vtg) mRNA. This mRNA was assayed using in situ hybridization (ISH) and real-time PCR. The results of this study suggest that flutamide treatment is capable of disrupting the production of the major vitellogenin isoform, Vtg3, in this species. The results of these studies reveal that flutamide induced a dose-dependent inhibition of vitellogenin (Vtg) mRNA in the testes of male Medaka fry. This effect was confirmed using real-time PCR. Finally, we have used the Medaka fry to evaluate the effects of other antiandrogens, such as cyproterone, on vitellogenin production. In summary, these results demonstrate that antiandrogens can have significant effects on testis development and function in male Medaka.
males per group were dosed orally once daily from PND 22 to 42/43. The five groups in Set A were dosed with 0 mg/kg/day in corn oil, 25 or 100 mg/kg/day CN, and 30 or 60 mg/kg/day DE 71. The three groups in Set B were dosed with 0 mg/kg/day in corn oil, and 12.5 or 50 mg/kg/day MET. Animals were examined for age and body weight at vaginal opening (VO) daily, beginning on PND 22; day of first estrous (DFE) and vaginal cytology. Necropsies were performed 2 hours post-dose on PND 42/43. Selected organs were weighed; ovaries, pituitary glands and thymuses were fixed for histological examination; and serum was collected for hormone analysis (thyroxine [T4] and thyroid-stimulating hormone [TSH]). There were no clinical signs of toxicity or effects on feed consumption during the study. There was a decrease in body weight and/or gain associated with CN and MET. Endocrine disruptive effects of: CN at 25 and/or 100 mg/kg/day included reduced organ weights of the ovaries and pituitary gland, a marked delay in VO and in the DFE, altered hyperprolactinemic hypertrophic uterine scores; decreased T4 and increased TSH. DE-71 at 30 and/or 60 mg/kg/day included reduced ovarian weight, increased thyroid weights; decreased thyroid colloid areas; decreased T4 and increased TSH; and delayed VO and DFE. MET at 12.5 and/or 50 mg/kg/day included reduced ovarian weight, advanced VO, irregular vaginal cytology (extended estrous). Conclusion: The applied experimental design satisfactorily detected the endocrine disruptive potential of the xenobiotics used in this validation.

2111 MALE PUBERTAL ASSAY: VALIDATION USING KNOWN ENDOCRINE DISRUPTORS.

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This study was performed to validate the ability of this assay to detect the endocrine disruptive potential of xenobiotics Vinclozolin (VN), DE 71, 1-Chloro-2-Nitrobenzene (CN) and Dibutyl Phthalate (DP) in pubertal male rats. Fourteen (Set A) and 15 (Set B) male rats were grouped orally once daily from PND 23 to 52/53. The 5 groups in Set A were dosed with 0 mg/kg/day in corn oil, 30 or 100 mg/kg/day VN, and 30 or 60 mg/kg/day DE 71. The 5 groups in Set B were dosed with 0 mg/kg/day in corn oil, 25 or 100 mg/kg/day CN, and 500 or 1000 mg/kg/day DP. Rats were examined for preputial separation (PS) daily from PND 30. Necropsies were done 2 hours post-dose on PND 52/53. Selected organs were weighed; testes, epididymides and thyroids were histologically evaluated; and serum was analyzed for hormones (thyroxine [T4], thyroid-stimulating hormone [TSH], and testosterone [T]). There were no clinical signs of toxicity or effects on feed consumption. There was a slight decrease in body weight gain from PND 23 to 52/53 associated with DE 71 and VN. Endocrine disruptive effects of: DP at 500 and/or 1000 mg/kg/day included decreased size and organ weights for the testes, epididymis and seminal vesicles (SV); reduced levator ani/bulbocavernous (LABC) muscle weight; severe seminiferous tubule atrophy with totally depleted germ cells; reduced T4, TSH and T levels; and a delay in PS. VN at 30 and/or 100 mg/kg/day included reduced epididymis, SV, and LABC muscle and increased testis weights; reduced T4 and increased T levels; and a delay in PS. CN at 100 mg/kg/day included reduced ventral prostate, SV, LABC muscle; reduced TSH and increased T levels; and a delay in PS. DE 71 at 30 and/or 60 mg/kg/day included reduced LABC muscle and increased testis weights; reduced T4 and T and increased TSH levels; increased thyroid epithelial cell height, decreased colloid area; and a slightly delayed PS. Conclusion: The applied experimental design satisfactorily detected the endocrine disruptive potential of the xenobiotics used in this validation.

2112 INTERACTION OF PERFLUOROALKYL ACIDS WITH HUMAN ESTROGEN RECEPTOR ALPHA.

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Previously, we reported that multiple perfluoralkyl acids (PFAAs) bind to the estrogen receptor (ER), induce expression of the estrogen biomarker protein vitellogenin and enhance liver carcinogenesis in rainbow trout, an animal model that represents the lack of sensitivity to peroxisome proliferators observed in humans. We now report new results from two studies examining the interaction of several PFAAs with human ERα. First, human embryonic kidney cells were transfected with the minimal SV40 promoter driving a luciferase gene containing the estrogen responsive element (ERE) and human ERα. Cells were then treated with estradiol (E2), perfluoroocanoic (PFOA), -nonanoic (PFNA), -decanoic (PFDA) and undecanoic (PFUnDA) acids as well as perfluorooctane sulfonate (PFOS) and 8:2 fluoro-tetradecanoic alcohol (FrOH). PFOA, PFNA, PFDA and PFOS all significantly inhibited gene reporter activity up to 2.5-fold at concentrations ranging from 10 to 1000 nM (PFOS increased reporter activity at 1 nM). PFUnDA and FrOH did not markedly alter gene reporter activity. Second, we employed a computational model using an iterative scoring method based upon 3D coordinates of the human ERα ligand binding domain complexed with E2 to investigate molecular docking of these putative ligands with the receptor. PFOA, PFNA, PFDA and PFOS all efficiently docked with hERα and formed a hydrogen bond at residue Arg394, similar to other known xenosterogens bisphenol A and nonylphenol. Interestingly, FrOH was found to effectively dock in this model similarly to the native hormone E2, which formed hydrogen bonds with both the Arg394 and Gln353 residues. PFUnDA did not interact with ERα in this computational model. These data support the contention that several PFAAs may be xenoestrogens. Supported by NIH grants ES07060, ES03850 and ES00210.

2113 THE ATRAZINE METABOLITE DACT CAUSES SUPPRESSION OF LH RELEASE IN LBT2 PITUITARY CELLS.

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Chlorotriazine (CI-TRI) compounds such as atrazine (ATRA) are among the most commonly used herbicides worldwide. Exposure to ATRA and other CI-TRIs causes a spectrum of reproductive and developmental deficits in laboratory species that involves selective disruption of the hypothalamic-pituitary-gonadal (HPG) axis and blunting of the surge of luteinizing hormone (LH) from the pituitary gland induced by gonadotropin releasing hormone (GnRH). While this effect is well known, the mechanism of action by which the chlorotriazines blunt the LH surge is not known. Calcium is known to play a central role in the secretion of LH from the pituitary gonadotrophs. Therefore, the objective of the present study was to elucidate if exposure to the atrazine metabolite diaminochlorotriazine (DACT) alters GnRH-induced intracellular Ca2+ release. LBT2 cells were treated for 24 hrs with 300uM DACT and imaged for intracellular calcium utilizing the fluorescent probe Fluo-4 AM. Images were captured every 2 sec and 10mM GnRH added to those cells. There was a slight decrease in the reduction in the GnRH-induced cytosolic Ca2+ transient. This decreased Ca2+ response correlated with loss of Ca2+ from thapsigargin-sensitive ER stores but could be recovered using the Ca2+ ionophore, A23187. Suppression of LH was also significantly reduced in DACT treated cells and was partially restored by treatment with A23187. These results suggest that DACT directly targets G protein-coupled Ca2+ release signals in LBT2 cells to inhibit GnRH-stimulated release of LH. This work was supported by a grant from the College of Veterinary Medicine.

2114 A 28-DAY TOXICITY STUDY IN JUVENILE BEAGLE DOGS WITH A ONE MONTH RECOVERY FOLLOWING ORAL ADMINISTRATION OF FAROPENEM MEDOXOMIL.

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Faropenem Medoxomil (FM) is an orally active beta-lactam antibiotic belonging to the penem group which is being developed for community antibiotic use. The objective of this study was to determine the toxicity of FM when administered to neonatal/juvenile dogs for 28 consecutive days. The study consisted of a vehicle control and four treatment groups of five male and female dogs/group (2-3 weeks of age at study initiation). The treatment groups received FM at dose levels of 100, 300, 600, or 1400 mg/kg/day (25, 75, 150, or 350 mg/kg/dose, respectively). FM or vehicle was administered to all groups via oral gavage, four times a day at a dose volume of 5 mL/kg/dose. Blood samples for clinical pathology evaluations were collected from all animals at pretest, during Week 2, and prior to the terminal (Week 4) and recovery necropsies. Urine samples were collected from all animals prior to terminal and recovery necropsies via cystocentesis. TK samples were collected from all animals at designated time points on Days 1 and 27. Following 28 days of administration, two animals/sex at 0 and 1400 mg/kg/day were maintained for a 1 month recovery period and then sacrificed with full necropsy. Body weight was significantly decreased in male puppies at 1400 mg/kg/day when compared to the control male animals. Comparison of Day 27 with Day 1 TK parameters showed a change in faropenem pharmacokinetic behavior over time with an apparent increase in the rate of clearance as observed in decrease in AUC(0-6) with little to no change in Cmax and a decrease in Tmax values. Kidney lesions consisting of minimal to mild vacuolation and tubular degeneration/necrosis were observed at 1400 mg/kg/day. Based on these results, the No Observed Adverse Effect Level (NOAEL) for general toxicity was 600 mg/kg/day based on kidney vacuolation and tubular degeneration/necrosis observed at 1400 mg/kg/day.
In the presence of foreign compounds, metabolic homeostasis of the organism is maintained by the liver’s ability to detoxify and eliminate xenobiotics. This is accomplished, in part, by the expression of xenobiotic metabolizing enzymes (XMEs), which metabolize xenobiotics and determine whether exposure will result in toxicity. The goal of this study was to identify XMEs that exhibit expression changes at different life stages including fetal, neonatal and the aged. We examined global expression changes using full-genome Affymetrix gene chips and confirmed changes with RT-PCR. The livers from untreated fetal mice (GD11.5-GD19), and to a lesser extent neonatal mice (PND10) exhibited dramatic differences compared to young adult animals including 1) suppression of phase I and II genes, 2) increased expression of a subset of phase III genes, 3) signatures of hematopoietic cells, especially nucleated erythrocytes and 4) a pancreas-like signature. A comparison of fetal and adult human livers from archived samples revealed similarities to the mouse results including 1) a general suppression of XME genes and 2) greater similarity of fetal liver to adult pancreas than adult liver. A comparison of the livers from young adult and old mice or humans revealed more subtle changes in gene expression including those in phase III transporters. In addition, we identified gene expression differences between men and women in the young or old groups, some of which were XMEs. These studies indicate that the livers from fetal or neonatal mice and humans exhibit dramatic changes in XMEs compared to young adults that may lead to differences in the metabolism of xenobiotics. (This abstract does not necessarily reflect U.S. EPA policy).

**2116 COMPARATIVE ANALYSIS OF CARDIAC RHYTHM AND QUANTITATIVE ELEMENTS OF THE ELECTROCARDIOGRAM (ECG) IN JUVENILE AND ADULT BEAGLE DOGS.**

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Drug therapies targeted for the human pediatric population now require testing in juvenile preclinical animal models to identify and characterize potential liabilities. The designs for these studies are based on FDA’s Guidance for Industry: Nonclinical Safety Evaluation of Pediatric Drug Products (February 2006) and EMEA’s Guideline on the Need for Nonclinical Testing in Juvenile Animals of Pharmaceuticals for Paediatric Indications (January 2008). One important function evaluated in juvenile large-animal studies is basic cardiac electrophysiology (via ECG). Due to the variability inherent in ECG determinations based on environment, technique, and other subject-specific factors (e.g., developmental stage), it is essential that research facilities develop/maintain appropriate test methodologies and historical control data. The purpose of this study was to utilize such datasets to compare standard ECG parameters obtained in juvenile and adult dogs. Based on recent literature describing differences in the developing cardiac conduction system in juvenile dogs, the issue of developmental differences is compelling, and the implications to drug therapy may be critical in appropriately defining the heart’s potential susceptibility during specific periods. Post-weaning beagle dogs were evaluated at 7 and 11 weeks of age using a modified 6-lead ECG system (Leads I, II, III, aVR, aVL, and aVF, recorded at 50 mm/sec). Comparative data indicate distinct differences in heart rate and ECG intervals sensitive to heart rate variability in juveniles, as compared to adults (BR ±1.7%, PR +1.9%, QRS -28.2%, QT -12.9%, QTc[Bazett] +13.1%). Fixed-factor QT correction procedures performed differently in adult and juvenile animals, with elevated heart rates in juveniles associated with even greater overestimation of QTc intervals. The present results confirm maturation differences in chronotropy, and associated changes in some indices of electrocardiographic cycling.

**2117 MITOCHONDRIAL TOXICITY OF TOBACCO IN SMOKING PREGNANT MOTHERS AND THEIR NEWBORN.**

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Background: Fetus exposed to tobacco presents a reduced intrauterine growth and weight at delivery of unknown etiology. It can be due to hypoxia induced by carbon monoxide (CO) binding to hemoglobin (COHb) or to the mitochondrial toxicity of this tobacco component that binds mitochondrial complex IV (mCIV) heme group. Methods: We have studied peripheral blood mononuclear cells (ficoI) of 7 smoking pregnant women and their newborns at delivery with respect 23 non-smoking control mothers and their newborns. Mitochondrial CIV activity, mitochondrial amount and oxidative stress were measured by spectrophotometry and mitochondrial DNA (mtDNA) levels by quantitative real-time PCR. Results: Enzymatic activity of mCIV, absolute or relative to mitochondrial amount, and mtDNA were decreased in smoking pregnant mothers, although non-significantly, with respect controls (28.21±5.51 vs. 54.77±9.59, 0.38±0.04 vs. 0.42±0.04 and 3.10±0.54 vs. 4.39±0.82, p<0.05). Absolute mCIV was significantly inhibited in their newborn (20.84±9.81 vs. 54.41±7.67, p<0.05), and showed a trend towards decrease when considered normalized to mitochondria (0.38±0.06 vs. 0.47±0.05, p<0.5). Mitochondrial DNA content also showed a trend to be reduced in those children (2.22±0.61 vs. 2.68±0.29, p<0.05) contrary to their increased oxidative stress levels (2.85±2.34 vs. 0.38±0.10, p<0.05). Mitochondrial complex IV activity in all studied mothers and newborns were significantly and positively correlated (p<0.005). Conclusions: Correlated mCIV dysfunction between smoking pregnant women and their newborns indicates common toxic etiology. Mitochondrial CIV inhibition could be accompanied by increased oxidative stress levels and decreased mtDNA amount. Although preliminary, these results show that the mitochondrial lesion induced by CO of tobacco smoke could explain reduced intrauterine growth and weight of newborn at delivery. Financial support: FIS 0229/08.

**2118 ACCURATE AND EFFICIENT ANALYSIS OF METALS IN CONSUMER PRODUCTS USING XRF TECHNOLOGY.**

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In the last few years we have learned that many consumer products contain undesirable amounts of toxic chemicals. This particularly serious for children, who are more vulnerable to exposure and the effects of these chemicals than adults. Some of the most hazardous of these chemicals are metals such as lead, cadmium, and mercury. The use of advanced technology, coupled with sophisticated software can quickly and efficiently provide precise, accurate assessment of metals in a product. The use of a handheld, X-Ray Fluorescence (XRF) analyzers can provide information about a variety of elements, including heavy metals, that may exist within a home or business: in consumer products; in soils; or in any other materials you have in the environment. Over the past two years, Environmental Services & Solutions (Esco Safety Check) has preformed tens of thousands of tests using XRF Analyzers and has compiled data on a variety of consumer products. The data collected includes: what type of product, if the product is new or used, what type of surface coating or substrate is present, and how the product may be used. Consumer products can contain a wide variety of heavy metals including lead, cadmium, arsenic and other potentially harmful elements. In our testing, we found total lead content over 300 PPM (the current federal regulation for children's products) in 6.81% of all tests. Cadmium content over 40 PPM was found in 3.64% of all tests. In general products that are used were found to contain higher quantities of lead than new products (10.15% vs. 4.42%) and Children's products were found to have below average levels of total lead content (4.05% over 300 PPM). However, 16.27% of mouthable products that children may use were found with contain lead in excess of 300 PPM. Of all defined substrates: Ceramics were found to contain lead and cadmium most often. 51.02% of tests on ceramics were found with lead in excess of 300 PPM and 12.03% with cadmium above 40 PPM. We present here data from a large number tests we have completed over the past year.
ate the feasibility of assessing respiratory function (tidal volume, minute volume and respiratory rate) as an indicator of lung development and direct pharmacology in juvenile animals. Respiratory function was assessed in conscious non-restrained Sprague-Dawley rats during a whole body plethysmograph. Assessments were conducted between Day 3 and 60 postpartum. Animals were acclimated for 15 minutes prior to conducting a 30 minute assessment, and were treated with known respiratory depressives, Baflofen, on Days 7, 14, 21 and 48 post partum which resulted in a reduction in respiratory rate and overall minute volume, confirming the model was sensitive enough to detect changes in respiratory function. These data confirm that safety pharmacology assessments can be adapted to meet the growing requirement for additional safety assessments on juvenile toxicity studies and that existing models can be tailored to provide sensitive assessments of both toxicological and pharmacological effects on respiratory function in juvenile rats.

2120 ATTENUATION OF HYPOXIA-INDUCED RETINOPATHY IN THE NEWBORN RAT MODEL BY β-NAPHTHOFLAVONE.

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Supplemental oxygen administration, which is frequently encountered in the treatment of premature infants suffering from respiratory distress, contributes to the development of retinopathy of prematurity (ROP). The purpose of this study was to test the hypothesis that exposure of newborn rats to a combination of β-naphtoflavone (BNF) and hypoxia would alleviate retinopathy and abnormal neovascularization compared to those exposed to hypoxia alone. Newborn Fisher 344 rats were maintained in room air or exposed to hypoxia (88% (gt) to 95% O2) for 7 days. Some animals were treated i.p. with BNF (40 mg/kg) or vehicle (saline), once daily for the first 4 days of hypoxic exposures. Animals were sacrificed at selected time points after termination of hypoxia. Retinal histopathology and vascular densities of flat mounted retinas were assessed, mRNA expression of VEGF and its receptors (VEGFR-1s/FLT-1 and VEGFR-2) was determined by real time RT-PCR. Immediately after 7 days of exposure to hypoxia alone or to hypoxia + BNF, we observed constricted retinal vessels, compared to air breathing animals. Seven to thirty days after termination of hypoxia, the animals displayed formation of abnormal retinal vessels and capillaries, whereas animals given BNF+ hypoxia showed significantly lesser extent of neovascularization. At these time points, VEGF mRNA expression in the hypoxia group was much higher than that of the BNF+ hypoxia group. On the other hand, the expression of VEGFR-1 and -FLT-1, but not VEGFR2, was upregulated by BNF + hypoxia, compared to the hypoxia only group. Our study supported the hypothesis that BNF protects retinas from oxygen-induced retinopathy with abnormal neovascularization, and that augmentation of the expression of VEGFR1 contributes to the retinoprotective effects of BNF. These results encourage development of further studies to elucidate molecular mechanisms of the protective action of BNF action, which could lead to clinical trials in infants who are at risk of developingROP.

2121 MITOCHONDRIAL DNA DEPLETION IN HIV-INFECTED CHILDREN.

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Background Both HIV infection and highly-active antiretroviral therapy (HAART) lead to mitochondrial (mt) dysfunction, a major pathway for some adverse events in HIV-infected adults. Little is known about mt toxicity in children. The aim of this study is to determine whether there exist alterations in mtDNA, mtRNA content and mitochondrial respiratory chain (MRC) function in peripheral-blood mononuclear cells (PBMCs) from HIV-infected children. Methods: A cross-sectional study in PBMCs was performed in 64 HIV-infected children (47 HAART-treated and 17 off treatment) and 25 healthy controls. We measured mtDNA and mtRNA content by Real-Time PCR. MRC enzymatic activity (complexes I, II-III, G3PDH, G3PDH-CIII, and CII function) was measured by spectrophotometry, and mitochondrial mass was estimated by cytrate synthase activity. Cytophrome-C-oxidase subunits of CIV and mt content were assessed by western-blots analysis. Results: A reduction in PBMCs mtDNA levels was observed in HIV-infected children compared to healthy controls (4.35±0.25 and 5.82±0.48, respectively; 25%, p< 0.005), together with similar levels of mtRNA (0.07±0.01 and 0.06±0.01, ps< 0.19) and protein subunit content. We found a reduction in CII+III+IV activity. Among HIV-infected children, mtDNA levels did not correlate with viral load. CD4 counts or percentages, and lactacemia at the time of assessment. No further differences were observed among treated patients when HAART-related variables (time on treatment, use of ddI or d4T, IF or NNRTI-based regimens) were considered. Conclusions: The observed depletion in mtDNA content in HIV-infected children did not lead to differences in mtRNA levels or most part of MRC function.

2122 BSEP INHIBITION AND RISK OF DRUG INDUCED LIVER INJURY IN MAN.

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To explore the relationship between inhibition of the bile salt efflux pump (BSEP/Bsep; ABCB11/Abcb11) and drug induced liver injury (DILI) in man, we have evaluated the BSEP inhibition by hepatic toxic and non hepatic marketed drugs in vitro, and explored functional consequences arising from Bsep inhibition in the rat in vivo. Inhibition of human BSEP and rat Bsep was quantified in plasma membrane vesicles prepared from transfected â21 insect cells using the probe substrate 3H-taurocholate, and in cultured rat hepatocytes by high content imaging using chollulyxysfluorescein as a probe substrate and a Cellomics Arrayscan™ algorithm. A high correlation was observed between potencies of inhibition of human BSEP and rat Bsep in vesicles (r=0.92), and between potency of rat Bsep inhibition in vesicles and hepatocytes (r=0.80). Potent inhibition of both BSEP/Bsep inhibition in vitro (cyclosporin, glibenclamide and troglitazone) caused elevated levels of plasma bile acids following oral dosing to rats, indicating functional transporter inhibition in vivo. We conclude that potent BSEP/Bsep inhibition by drugs correlates with increased risk of cholestatic drug hepatotoxicity in man, and that in vitro evaluation of this liability is useful for DILI hazard identification. Our results also suggest that evaluation of plasma bile acid levels in vivo may add additional value, and aid in risk assessment.

2123 TROVAFLOXacin AND TUMOR NECROSIS FACTOR-α INTERACT TO CAUSE CELL DEATH IN HEPG2 CELLS.

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Trovaloxacin (TVX) is a fluoroquinolone antibiotic, the use of which was severely restricted after it caused idiosyncratic hepatotoxicity in humans. Previous studies showed that cotreatment of mice with TVX and a modest inflammatory stress induced by tumor necrosis factor-α (TNF-α) caused liver injury, whereas neither TVX nor TNF-α alone resulted in liver damage. An in vitro model was developed using the HepG2 human hepatoma cell line to characterize mechanisms by which TVX and TNF-α interact to cause liver injury. Various concentrations of TVX (1.25-20 μM) and TNF-α (0.003-15 ng/mL) were applied simultaneously to HepG2 cells in culture, and cytotoxicity was evaluated. At the concentrations tested, neither TVX nor TNF-α alone elicited cell death; however, in the presence of TNF-α (0.015 ng/mL – 15 ng/mL) TVX produced concentration-dependent cytotoxicity. Cotreatment with 20 μM TVX and 3.9 ng/mL TNF-α generated significant cytotoxicity and was selected for further study. Cytotoxicity began approximately 8 hours after TVX/TNF-α addition and increased with time. In mice, TVX/TNF-α treatment resulted in apoptotic as well as oncocytic hepato cellular necrosis (Shaw et al., J. Pharmacol. Exp. Ther. 330: 72, 2009). In HepG2 cells, application of a pan caspase inhibitor (Z-VAD-FMK) protected TVX/TNF-α treatment from inducing apoptosis as well as oncocytic hepato cellular necrosis. At these time points, expression of the pro-apoptotic Bax molecule and its receptors (VEGFR-1/sFLT-1 and VEGFR-2) was determined by real time PCR. Neovascularization of flat mounted retinas were assessed. mRNA expression of VEGFR-1 and -2 (cyclosporin, glibenclamide and troglitazone) caused elevated levels of plasma bile acids following oral dosing to rats, indicating functional transporter inhibition in vivo. We conclude that potent BSEP/Bsep inhibition by drugs correlates with increased risk of cholestatic drug hepatotoxicity in man, and that in vitro evaluation of this liability is useful for DILI hazard identification. Our results also suggest that evaluation of plasma bile acid levels in vivo may add additional value, and aid in risk assessment.

2124 TOLERANCE IN AN ANIMAL MODEL OF AMODIAQUINE-INDUCED LIVER INJURY.

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Background: Amodiaquine (AQ) causes idiosyncratic liver failure and agranulocytosis. The purpose of this study is to establish an animal model of idiosyncratic AQ-induced liver injury. AQ had previously been reported to induce anti-AQ antibodies after treatment of rats for 4 days at a dose of 125 mg/kg/day. However, we found that this dose caused generalized toxicity after a longer period of treatment. Methods and Results: BN rats were treated with AQ 62.5 mg/kg, 6 day/week, by
2125 TROVAFLOXACIN POTENTIATES LPS-INDUCED TNFα EXPRESSION IN A MACROPHAGE CELL LINE.

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Trovafloxacin (TVX), a quinolone antibiotic, has been associated with idiosyncratic hepatotoxicity in patients. Previous results indicated that the combination of non-toxic doses of lipopolysaccharide (LPS) and TVX resulted in elevated serum ALT hepatotoxicity in patients. Previous results indicated that the combination of non-toxic doses of lipopolysaccharide (LPS) and TVX resulted in elevated serum ALT. This study was performed to develop an in vitro model of TVX and LPS interaction. A murine macrophage cell line (RAW 264.7) was treated with various concentrations of TVX (1–100 μM) and LPS (0.1–100 ng/ml). Cotreatment resulted in significantly greater TNFα protein concentration compared with TVX or LPS treatment alone. The increase occurred 3–10 h after LPS treatment. In contrast, levofloxacin, a quinolone without idiosyncratic liability in humans, had no significant effect on LPS-induced TNFα concentration. Real-time PCR analysis revealed a selective increase in mRNA for TNFα, ECR-1, IL-1β and IL-10, by TVX/LPS cotreatment. This suggests a role for p38 MAP kinase and NF-κB in TVX/LPS interaction in macrophages. (Supported by NIH grant RO1DK061315).

2126 ASSESS THE SEVERITY OF DILI USING HIGH CONTENT SCREENING ASSAY BASED ON RAT PRIMARY HEPATOCYTES.


In this study, we tested the hypothesis that rat primary hepatocytes applied to high-content screening assay could be used as an in-vitro model to predict the severe drug-induced liver injury (DILI) incidence of pharmaceuticals. Using a library of 136 drugs agents as a training set and another 44 drugs as a testing set, we developed a classifier based on safety index obtained through a panel of cytoxicity parameters to predict the severe DILI outcome of pharmaceuticals. It was found that cell loss, nuclear size, DNA damage, apoptosis, lysosomal mass, and steatosis, measured by the high-content imaging technology, were the most informative parameters in the panel, and 24 and 48hr were the appropriate drug incubation time. Safety index of 100 was determined to significantly associate with the potential to induce severe DILI based on the training set. Furthermore, our new approach to predict the severe DILI of pharmaceuticals has obtained 65% sensitivity and 84% specificity in the training set, and 58% sensitivity and 75% specificity in the testing set, considering both 30% stress and 50% animal toxicity studies (Olson, Betton et al. 2000), which suggests that it could be a promising in-vitro model for testing severe DILI outcome of pharmaceuticals.

2127 POTENTIAL HEPATOTOXICITY OF 5-(3,5-DICHLOROPHENYMETHYL)-2,4-ThIAZOLIDINEDIONE (5-DCPMT) IN RATS.


The thiazolidinedione (TZD) ring is a constituent of the glitazones, a group of drugs used in the treatment of type II diabetes. Several cases of liver injury have been reported following chronic use of these drugs. To further investigate TZD ring-induced hepatotoxicity, 5-(3,5-dichlorophenylmethyl)-2,4-thiazolidinedione (5-DCPMT), a compound with a similar substitution pattern to the insulin-sensitizing drugs, was synthesized. Male, Fischer 344 rats were administered 5-DCPMT (1.0, 0.6, 0.4, and 0.2 mg/kg/d) ip in corn oil. Control animals received corn oil (4 mL/kg) only. Of the four rats receiving the 1.0 mg/kg dose of 5-DCPMT, two died within minutes of receiving the injection. Liver and kidney function and morphology were assessed at 24 hrs. Serum alanine aminotransferase (ALT) levels were not significantly different from controls. Liver weight was significantly increased in rats receiving 0.2 mg/kg 5-DCPMT. Morphologically, livers showed areas of irregular staining, suggesting metabolic changes within the cells, extending from the centrilobular vein to the midzonal region. There were also diffuse areas of cells showing karyolysis and pyknosis. No inflammatory cells were observed in any of the lesions. Both blood urea nitrogen (BUN) levels and kidney weights were significantly increased in rats receiving 0.6 mg/kg 5-DCPMT. Urine protein was significantly increased in rats receiving 0.4 mg/kg 5-DCPMT. With the exception of slight proximal tubule cell swelling, there was no evidence of kidney damage in any of the treatment groups. In conclusion, morphological results suggest that 5-DCPMT produces moderate liver damage that is consistent with changes associated with apoptotic; however, this will require further investigation. Supported in part by PHS grant ES012499.

2128 CORRELATION OF TOXICITY INDUCED ENDOGENOUS METABOLITES DISREGULATION WITH XENOBIOIC METABOLISM BY AN INTEGRATED METABONOMIC APPROACH: EFFECTS OF LIVER-SPECIFIC KNOCKOUT OF THE NADPH-CYTOCHROME P450 REDUCTASE GENE ON TRIPTOYLDE INDUCED TOXICITY.

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Triptolide, the primary active component of the traditional Chinese medicine Tripterygium wilfordii Hook F, has a wide range of pharmacological activities. However, its clinical use has been limited by its high toxicity. This study investigated the effects of P450 metabolism on triptolide induced toxicity using liver-specific NADPH-cytochrome P450 reductase (CPR) knockout mice. Our results indicated that triptolide can cause injuries in many organs, such as liver, kidney, spleen. Loss of hepatic P450 activity influenced the pharmacokinetics and distribution of triptolide in vivo and increased the risk of triptolide induced toxicity. The metabolism change of triptolide by hepatic P450 further induced a series of metabolism disturbance. In conclusion, our findings suggest that the hepatic P450 enzymes play a crucial role in the metabolic detoxification of triptolide. When applying triptolide in clinic, P450 inhibition related drug-drug interaction should be avoided.

2129 INCREASED MITOCHONDRIAL PEROXYNITRITE STRESS AND HEPATIC MITOCHONDRIAL INJURY IN HETEROZYGOUS Sod2−/− MICE EXPOSED TO TROVAFLOXACIN.

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Trovafloxacin (TVX), a fluoroquinolone antibiotic, has been associated with rare idiosyncratic hepatotoxicity that led to the withdrawal of the drug. The underlying mechanisms of TVX-induced liver injury in susceptible patients are, however, largely unknown. The aim of this study was to explore whether TVX increases hepatic peroxynitrite stress in heterozygous Sod2−/− mice, a murine model of underlying human mitochondrial disorders commonly featuring increased mitochondrial oxidative stress. Both wild-type and mutant mice were given ala-TVX, the water soluble prodrug of TVX (0, 11, or 33 mg/kg/day, ip) for 4 weeks. We found that ala-TVX caused selective mitochondrial protein modification with increased nitrotyrosine content and decreased mitochondrial aconitate and complex I activity in Sod2−/− mice, consistent with increased peroxynitrite formation. To explore whether TVX might stimulate mitochondrial nitric oxide (NO) generation, immortalized human hepatocytes were exposed to ala-TVX and loaded with the NO-selective fluorescent probe, DAF-2-DA. NO-derived fluorescence co-localized with Mitotracker Red in mitochondria, suggesting activation of the Ca2+-stimulated mitochondrial nitric oxide synthase. The expression of cytochrome c oxidase subunit 2 (mtDNA-encoded) was selectively down-regulated after ala-TVX treatment in Sod2−/−, but not wild-type, mice. In contrast, the transcript levels of a number of genes encoding antioxidant enzymes was decreased in Sod2−/− mice.

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Acetaminophen (APAP) overdose can cause acute liver failure in patients and hepato-toxicity in mouse models, but mechanism-based treatment to rescue patients from liver failure is still limited. Although it has become clear that the thiol-reactive intermediate, NAPQI, causes mitochondria-mediated hepatocellular necrosis, the mechanisms leading to APAP-induced mitochondrial outer membrane permeabilization have remained unclear. By elucidating this mechanism further it may be possible to find therapeutic targets which could be useful in treating APAP overdose. Previous studies have demonstrated that cyclosporin A (CsA), an inhibitor of the mPT pore, protects from APAP hepatotoxicity in mice, suggesting that cytochrome D (CyD), a component of the regulated mPT and CsA-binding protein, might play a key role. In this study, we used Ppif-null (CyD-deficient) mice to define the role of the regulated mPT in APAP hepatotoxicity in vivo. APAP (600 mg/kg, ip, in Soltol HS-15) was administered to Ppif+/+, Ppif-/−, and Ppif-/− mice. Control studies had revealed that Soltol HS-15 itself did not inhibit APAP bioactivation. After 24 h, marked centrifugal necrosis and similar increases in serum ALT activity were observed in both genotypes. To explore the role of chemical inhibition of CyD, CyD was administered (600 mg/kg, i.p.) followed by injection of a non-cholostatic dose of Ca²⁺ (10 mg/kg, i.p.) 1.5 h post-dose. Ca²⁺ afforded significant protection in wild-type, but not Ppif-/−, mice. Because Ca²⁺ also binds to cytosolic cytochrome A, which activates calcineurin, we used tacrolimus (10 mg/kg, ip), which does not bind to mitochondrial CyD. We found that serum ALT activity was similar in both the wild-type and knock-out mice, supporting the hypothesis that T cells do not play an apparent role in APAP hepatotoxicity. These data demonstrate that both the regulated, Ca²⁺-sensitive mode of the mPT, as well as the unregulated mode, are involved in APAP hepatotoxicity in vivo, and that Ca²⁺ is unlikely to act through non-CyD-mediated pathways.

Liver breast cancer resistance protein (Bcrp, Abcg2) is a canalicular efflux transporter that mediates the biliary excretion of many anticancer agents, including mitoxantrone and topotecan. Bcrp also transports sulfate conjugates such as acetaminophen (APAP)-sulfate. Although APAP-sulfate is a non-toxic metabolite, the absence of Bcrp may impair biliary elimination of endogenous molecules that could alter the susceptibility of the liver to drug hepatotoxicity. In the present study, C57BL/6J and Bcrp−/- mice received 600mg/kg acetaminophen (APAP), i.p. Liver, kidneys and plasma were collected 24 or 48 hrs later. Analysis of plasma alanine aminotransferase (ALT) activity indicates that Bcrp−/- mice are more sensitive to liver injury by APAP. Wild-type mice showed 24hr ALT levels of approximately 1000U/L, while Bcrp−/- mice had values of 2000U/L. Bcrp−/- mice showed reduced ability to recover from liver injury, since ALT values were still significantly elevated at 48 hrs in comparison to wild-type mice. Blood urea nitrogen (BUN) was also measured as a marker of kidney injury. 24 hrs after APAP dosing, wild-type and Bcrp−/- mice had significantly higher BUN values than controls, with no significant differences between genotypes (approximately 60U/L). At 48 hrs, BUN values were further increased to 100U/L in Bcrp−/- mice, but not in wild-type mice. Collectively, these results indicate that mice lacking Bcrp are more susceptible to APAP hepatotoxicity and that they have an altered capacity to recover from both liver and kidney injury. Analysis of control livers from wild-type and Bcrp−/- mice showed no significant differences in protein levels of Cyp1a2, Cyp2e1, Cyp3a11 or Ugt1a6, suggesting that this differential susceptibility to liver and kidney toxicity is not due to variations in hepatic APAP metabolism. This work was supported by The National Institutes of Health Grant DK060557.

Oxidative stress and mitochondrial dysfunction play an important role in acetaminophen (APAP)-induced hepatocyte cell death. However, exact mechanisms involved in the process are controversial, in part, due to the disparity in findings between in vitro & in vivo studies. A major difference in this context is the oxygen tension, with cells in culture being exposed to 21% oxygen while those in the liver experience a gradient from 5-11% oxygen. To determine if oxygen tension could modulate hepatocyte responses to APAP, this study examined cells under normoxia (21% O₂), 10% O₂ & 5% O₂. Isolated primary mouse hepatocytes were treated with 5mM APAP for 15 hours under various oxygen tensions and cell death as assessed by LDH release. Mitochondrial function and membrane potential were assessed at 6 & 15h using the XTT assay and JC-1 fluorescence, respectively. Total mitochondrial reactive oxygen species as well as superoxide were measured using dihydroethodamine (DHR) & Mitosox Red® fluorescence, respectively. Exposure of hepatocytes to 5mM APAP for 15 hours at 21% O₂ resulted in significant cell death, accompanied by deterioration of mitochondrial function and loss of mitochondrial membrane potential, which was evident at 6 hours of treatment. Mitochondrial generation of superoxide was also elevated at 6 hours and by 15 hours an increase in DHR fluorescence was evident. Culture of cells at 5 & 10% O₂ resulted in significant protection against cell death. Mitochondrial function and membrane potential were better preserved at 6 hours, though the protective effect decreased by 15 hours. Culturing cells at 10% O₂ also prevented the increase in DHR fluorescence. Exposed cells had a heightened response to oxidative stress after exposure to APAP, despite having increased liver GSH content. This work was supported by The National Institutes of Health Grant DK060557.
Acetaminophen (APAP) overdose is the leading cause of acute liver failure in the US. We have demonstrated in mice that APAP causes fibrin deposition and increases plasminogen activator inhibitor-1 (PAI-1), an inhibitor of fibrinolysis. Inhibition of the hemostatic system with heparin attenuates liver injury, indicating a role in the pathogenesis. APAP treatment caused an increase in nuclear translocation of hypoxia-inducible factor 1α (HIF-1α) in livers (James et al., 2006), and HIF-1α can regulate PAI-1 production. We tested the hypothesis that APAP-induced hemostatic system activation is mediated through HIF-1α signaling. HIF-1α−/− and UBC-Cre-ERT2 mice were mated to generate UBC-Cre-ERT2/HIF-1αΔ/Δ mice capable of conditional deletion in the floxed HIF-1α gene when treated with tamoxifen (TAM). Male mice were treated with 200 mg/g body weight TAM or corn oil for 5 days to generate HIF-1αΔ/Δ (HIF-1α deficient) or HIF-1αΔ/Δ (HIF-1α sufficient) mice. 21 days later, mice were treated with saline or 400 mg/kg APAP, and liver and plasma samples were taken at 2 and 6 hours. HIF-1αΔ/Δ mice had modest liver injury at 2 h (750 U/L ALT) that progressed to severe liver injury by 6 h (6400 U/L ALT) characterized by centrilobular necrosis. HIF-1αΔ/Δ mice were protected from APAP hepatotoxicity (125 U/L ALT), and livers were free of necrosis at 6 h, suggesting that HIF-1α signaling is involved in APAP hepatotoxicity. APAP treatment increased fibrin deposition in the livers of HIF-1αΔ/Δ mice, and this was significantly attenuated in HIF-1α−/− mice. Active PAI-1 was elevated in plasma 6 h after APAP (4.5 mg/mL) in HIF-1αΔ/Δ mice compared to HIF-1α−/− mice (1.5 mg/mL). Taken together, the results suggest that HIF-1α is a mediator of the impaired fibrinolysis and hepatotoxicity associated with APAP toxicity. (Supported by NIH grant ES004139.)

Acetaminophen (APAP) is the leading cause of drug induced liver disease in the United States. S-adenosyl-L-methionine (SAMe) has previously been demonstrated by our lab and other investigators to reduce APAP hepatic toxicity. S-adenosyl-l-methionine (SAMe) is the principle biological methyl donor participating in the methylation of nucleic acids, proteins, and phospholipids. SAMe is a precursor for the transsulfuration pathway and formation of glutathione. Thus SAMe is critical for growth and recovery of the liver following APAP overdose. The present study
sought to examine alterations in hepatic SAMe subcellular levels in mitochondria and nuclei as well as alterations in metabolism by cytochrome P450 2E1 (CYP2E1) and ornithine decarboxylase (ODC) associated with APAP overdose. Male C57BL/6 mice (15-25 g) were randomly allocated between the following groups (n=5/group) and injected intraperitoneal with: Vehicle (15 ml/kg water), SAMe (1.25 mmol/kg), APAP (250 mg/kg), and SAMe administered 1-hr after APAP (SAMe + APAP). Livers were collected at 2, 4, or 6-hr following APAP administration. Hepatic subcellular levels of SAMe were determined by HPLC analysis. Mitochondrial SAMe levels were significantly decreased only in APAP and SAMe + APAP groups. At 2-hr following APAP overdose only the APAP group demonstrated a depression in CYP2E1 activity which was reversed by SAMe. In summary, APAP overdose depleted critical subcellular levels of mitochondrial and nuclear SAMe which would be anticipated to have an adverse influence on hepatic function. The current study provides insight into the mechanism by which SAMe is able to protect against APAP toxicity. (Supported by WV-NASA Space Consortium).

**2139 ROLE OF INTERLEUKIN-6 IN OZONE EXCERCIATION OF ACETAMINOPHEN-INDUCED LIVER INJURY.**

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We have reported that inhalation of ozone (O3), a common air pollutant, exacerbates acetaminophen (APAP)-induced liver injury in mice. Coexposed mice have greater hepatocellular necrosis and plasma alanine aminotransferase (ALT) activity compared to APAP-treated mice exposed only to filtered air. In addition, O3 caused significant reductions in hepatic cell proliferation and IL-6 production necessary for liver repair after APAP-induced injury. Since IL-6 is known to be an important factor in liver regeneration, the present study was designed to investigate its role in APAP/O3-induced liver injury and repair. We hypothesized that the lack of induction of hepatic IL-6 might be responsible for impaired hepatic repair and increased toxicity in coexposed mice. Fasted IL-6 sufficient and deficient male mice were given APAP (300 mg/kg ip) or saline vehicle alone. Two hours after treatment, mice were exposed by inhalation to 0 or 0.5 ppm O3 for 6h. Mice were sacrificed 24h after O3 exposure (32h after APAP). Hepatocellular proliferation (increased DNA synthesis) was assessed immunohistochromically using bromodeoxyuridine. The magnitude of liver injury was assessed by morphometric analysis of hepatocellular necrosis and ALT activity. Liver injury induced by APAP was similar in both IL-6 sufficient and deficient mice. Regenerative hepatocellular proliferation was elevated only in IL-6 sufficient mice exposed to APAP alone. O3 exposure inhibited the increase in hepatocellular proliferation after APAP injury similar to that observed in APAP-treated IL-6 deficient mice. These results suggest that 1) O3 exacerbation of APAP-induced liver injury is not IL-6 dependent and 2) the O3 impairment of reparative hepatocellular proliferation may be mediated by an inhibition of increased hepatic IL-6 production which is necessary for repair. Supported by NIH ES01617 and NIH ES004139.

**2140 ROLE OF GABA RECEPTORS IN THE ANTINOCESSION OF GABAPENTIN AND TRAMADOL.**

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Rational combinations of analgesics with different mechanisms of action can achieve improved analgesia and reduced toxicity. In previous work, we found that gabapentin significantly enhances antinociception of tramadol, an atypical opioid analgesic, and reduces its adverse effects in mice. Gabapentin, a novel anticonvulsant and an adjuvant analgesic, is a γ-aminobutyric acid (GABA) derivative. How gabapentin potentiates tramadol antinociception remains unclear. GABA receptors are well known to modulate pain transmission and perception. This study is to determine whether GABA receptors play a role in the antinociception of gabapentin and tramadol as well as their synergistic effects. Experiments were conducted in NIH Swiss female mice. Tramadol (T; 60 mg/kg) was intraperitoneally injected to mice. Gabapentin (G; 75 mg/kg) was per orally administered to mice 30 min before tramadol injection. GABA<sub>A</sub> receptor antagonist bicuculline (Bb; 2 mg/kg) or GABA<sub>B</sub> receptor antagonist 2-hydroxyasaclofen (H; 3 mg/kg) were subcutaneously injected to mice 15 min before gabapentin administration. Mice were randomly assigned into 6 groups, including T, G+T, H+T, B+T, H+G+T, and B+G+T groups. The latency of tail-flick response to radiant heat was measured before and after drug administration at different time intervals and area under the curve (AUC) was calculated. The AUCs for different groups were: T: 1456±25; G+T: 1718±60 (P<0.05 when compared to T); H+T: 1557±39 (P<0.05 when compared to T); B+T: 1563±66; H+G+T: 1718±109 and B+G+T: 1759±85. The results indicated that bicuculline had no effect on tramadol (alone or in combination with gabapentin)-produced antinociception. 2-Hydroxyasaclofen reduced tramadol antinociception but did not alter the antinociceptive synergistic effect of gabapentin on tramadol. In summary, blockade of GABA<sub>B</sub> receptor attenuates tramadol antinociception but does not affect the synergy of gabapentin and tramadol. GABA<sub>B</sub> receptor seems not to be involved in modulation of antinociception induced by tramadol, alone or in combination with gabapentin.

**2141 HUMAN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR mRNA AND PROTEIN EXPRESSION DURING DEVELOPMENT.**


Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that regulate energy homeostasis and are important in reproduction and development. PPARs can be activated by a wide variety of chemicals including pharmaceuticals and environmental contaminants. Studies have characterized the expression of the three PPAR subtypes (α, β, and γ) in rodents, but little is known about PPAR expression during human development. In this study, human adrenal, intestine, kidney, liver, lung, spleen, stomach, and thymus from gestational day 54-120 fetuses were obtained from the Birth Defects Research Laboratory at the University of Washington, Seattle. Quantitative PCR and Western blot analysis were used to evaluate mRNA and protein expression. In general, all PPAR subtypes were detected in all organs and expression appeared stable. Relative expression of PPARα, β, and γ varied by tissue. No significant changes in mRNA expression were observed for PPARα or PPARγ due to gestational age, but significant decreases in PPARβ message were seen in heart and intestine with age. The abundance of fetal PPAR mRNA was also compared to adult human samples (First Choice Human Total RNA, Ambion, Inc.). Generally, mRNA levels were either similar to adult or lower in the fetus. In liver and intestine, fetal and adult PPAR expression was the same, while in stomach and heart all three subtypes had lower fetal expression. Lower levels of fetal mRNA were also observed for PPARα and PPARβ in kidney and spleen, and for PPARγ in lung. Western blotting showed no changes with age in the heart, spleen, stomach or thymus for any PPAR subtype. Decreasing protein with age occurred for PPARα in kidney and lung and for PPARγ in kidney. However, with increasing age, higher levels of PPARα were found in intestine and PPARβ in liver and intestine. This study provides new information regarding the expression of PPAR subtypes in the human fetus, which will be instrumental in evaluation of PPAR responses in developing humans. (This abstract does not necessarily reflect U.S. EPA policy.)

**2142 PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-β/δ (PPAR β/δ) INHIBITS VIRAL HRAS1 (V- HRAS1)-INDUCED NEOPLASTIC TRANSFORMATION OF MOUSE PRIMARY KERATOCYTES.**

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Ligand activation of Ppar<sub>β</sub>/<sup>δ</sup> inhibits chemically-induced skin tumorigenesis. Since the oncogenic mutation of Harvey sarcoma ras virus gene (v-Hras1) is a critical event in chemical carcinogenesis, the role of Ppar<sub>β</sub>/<sup>δ</sup> in v-Hras1-induced neoplastic transformation was examined in mouse primary keratocytes. While v-Hras1 infected Ppar<sub>β</sub>/<sup>δ</sup>-null keratocytes exhibited increased cell proliferation in both low calcium and high calcium medium, ligand activation of Ppar<sub>β</sub>/<sup>δ</sup> decreased cell proliferation in wild-type keratocytes only in low calcium medium, which was characterized by a decrease of cells in S phase and an increase of cells in G2/M phase. The mRNA and protein levels of v-Hras1 oncogene were reduced in wild-type keratocytes by ligand activation of Ppar<sub>β</sub>/<sup>δ</sup> following 3 days of culture in low calcium medium and this effect was not found in similarly treated Ppar<sub>β</sub>/<sup>δ</sup>-null keratocytes. This decrease v-Hras1 expression was due to a negative selection against cells expressing higher levels of the v-Hras1 oncogene by ligand activation of Ppar<sub>β</sub>/<sup>δ</sup> in wild-type keratocytes. Interestingly, Ppar<sub>β</sub>/<sup>δ</sup>-null keratocytes
showed an enhanced endoplasmic reticulum (ER) stress 5 days post v-Hras1 infection, characterized by enhanced vacuolation and higher levels of several ER stress markers. Surprisingly, this increased level of ER stress did not lead to an increase in oncogene-induced senescence in Pparβ/δ-null keratinocytes, but rather, Pparβ/δ-null keratinocytes exhibited a lower level of cellular senescence compared to wild-type keratinocytes. Further, ligand activation of PPARβ/δ induced terminal differentiation in v-Hras1-infected wild-type keratinocytes and this effect was not observed in v-Hras1-infected Pparβ/δ-null keratinocytes. These results suggest that PPARβ/δ attenuates oncogenic-dependent oncoplastic transformation by inhibiting Hras1 signaling in keratinocytes. (Supported by CA124533, CA128826)

2144 FUNCTIONAL EXAMINATION OF THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-β/δ (PPARβ/δ) IN HUMAN COLON CANCER.

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The role of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) remains controversial. While some evidence suggests that activating PPARβ/δ promotes tumorigenesis, other reports suggest that activating PPARβ/δ has no effect or attenuates tumorigenesis. The latter observations are also supported by the well-established roles of PPARβ/δ in regulating glucose and lipid homeostasis and promoting cellular differentiation. Previous work has suggested that PPARβ/δ is up-regulated by the APC/β-catenin pathway in colon cancer and that the chemopreventive effects of non-steroidal anti-inflammatory drugs (NSAIDs) are mediated by repression of PPARβ/δ expression and function. However, more comprehensive analysis of human colon cancer cell lines with mutations in the APC/β-catenin pathway indicates that PPARβ/δ expression is not up-regulated by increased β-catenin-dependent activity. Further, increased expression of PPARβ/δ and function was also noted in human colon cancer cell lines treated with NSAIDs. Thus, there is a clear need for more quantitative analysis of PPARβ/δ function in colon cancer models. The present study examined expression of PPARβ/δ mRNA and protein in normal and cancerous human colon tissue. Colony formation assays using human colon cancer cell lines with NSAIDs was associated with increased expression of PPARβ/δ and activating PPARβ/δ in the presence of NSAIDs either enhanced or had no effect on cleaved poly (ADP-ribose) polymerase (PARP). Collectively, results from these studies provide additional evidence demonstrating the ligand activation of PPARβ/δ either inhibits cell proliferation or has little effect in human colon cancer models. (Supported by CA124533, CA128826)

2145 DIELDRIN INDUCES THE RAT PARAOXONASE (PON1) PROMOTER VIA PXR, RXR.

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Measurable levels of the legacy organochlorine insecticide, dieldrin, are still in soil and accumulation in exposed individuals is a possibility. Paraoxonase (PON1) is named for its ability to hydrolyze paraoxon, the active metabolite of the insecticide parathion, and is associated with HDL particles. In addition, PON1 has been inversely related to the prevalence of atherosclerotic cardiovascular disease. In vivo rat studies have shown that dieldrin can increase paraoxonase activity but the mechanism is unclear. Nuclear receptors can increase pesticide metabolism, and putative binding sites for PXR exist in the PON1 promoter. The present work examined the effect of dieldrin and nuclear receptors on PON1 promoter activity via the Dual-GloTM Luciferase Assay System. Expression constructs were created containing rat liver PXR only, RXR only, and PXR-RXR combined on the same vector. A competitor with the human PXR gene (SXR) was also obtained. The rat liver PON1 promoter was subcloned in front of the Renilla gene. Plasmids were transiently transfected, either alone or in combination, into the rat hepatoma cell line, McA-RH7777. Controls included the empty expression vector and a transfection control plasmid ready with Rh plasmids were transfected in the absence and in the presence of 10 μM 100 μM significantly increased (P<0.05) PON1 promoter activity in the presence of PXR or RXR alone and when PXR plus RXR were on the same vector. SXR alone, SXR or PXR plus RXR on separate vectors, and the control plasmids all failed to significantly induce PON1 promoter activity. The present study demonstrated that PXR may be as important as PXR in the dieldrin induction of the PON1 promoter and that human SXR and rat PXR are not functionally identical. To our knowledge, this is the first reported evidence of an interaction between PXR and the PON1 promoter.

2146 NATURALLY OCCURRING HUMAN CONSTITUTIVE ANDROSTENES RECEPTOR SPLICE VARIANTS UNDERGO LIGAND SELECTIVE PROTEIN INTERACTIONS.

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Modulation of gene transcriptional activities by nuclear receptors involves a complex interplay of numerous proteins that have diverse functions. The constitutive androstane receptor (CAR1) and its two splice variants, CAR2 and CAR3, are known to dimerize with the retinoid X receptor alpha (RXRα) for transcriptional activation. Previous studies have indicated that CAR1 does not require ligand binding for interaction with RXRα; however, it is unclear whether ligand is required for CAR2 or CAR3/RXRα heterodimerization. To investigate the mechanisms of CAR activation and heterodimerization, we conducted mammalian two-hybrid assays. COS-1 cells were co-transfected with the GAL4 DNA binding domain (DBD) domain PM-CAR and the GAL4 transcriptional activation domain VP16-RXRα vectors and assayed for GAL4-promoter mediated reporter gene activation following treatment with DMSO or selective CAR ligands. In cells co-transfected with CAR1 and RXRα, robust levels of reporter gene expression were detected in both the presence and absence of CITCO, the pan-CAR ligand, suggesting that CAR1 interacts readily with RXRα, even in the absence of ligand. In contrast, the interaction of CAR2 and CAR3 with RXRα required the presence of ligand. For CAR2, both the pan-CAR ligand, as well as the CAR2 specific ligand, bis-(2-ethyhexyl)phthalate (DEHP), were capable of triggering this interaction. Similarly, CITCO was required for CAR3 dimerization with RXRα. The results of these studies suggest that ligand binding to both CAR2 and CAR3 activates a conformational change in these receptors that subsequently enables RXRα interaction, whereas this structural alteration is not required for CAR1. Ongoing studies are aimed at determining the dynamics of dimerization and nuclear coregulator recruitment among the variant CARs.
A SINGLE AMINO ACID CONTROLS THE FUNCTIONAL SWITCH OF HUMAN CAR1 TO THE XENOBIOTIC ACTIVATED SPlicing VARIANT CAR3.

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The constitutive androstane receptor (CAR) is constitutively activated in immortalized cell lines independent of xenobiotic stimuli. This feature of CAR has limited its use as a sensor for xenobiotic-induced expression of drug-metabolizing enzymes. Recent reports, however, revealed that a splicing variant of human CAR (hCAR3), which contains an insertion of 5 amino acids, exhibits low basal but xenobiotic inducible activities in cell-based reporter assays. Nonetheless, the underlying mechanisms of this functional shift are not well understood. We have now generated chimeric constructs containing various residues of the 5 amino acids of hCAR3 and examined their response to typical hCAR activators. Our results showed that the retention of alanine (hCAR1+A) alone is sufficient to confine constitutively activated hCAR1 to the xenobiotic sensitive hCAR3. Notably, hCAR1+A was significantly activated by a series of known hCAR activators, and displayed activation superior to that of hCAR3. Moreover, intracellular localization assays revealed that hCAR1+A exhibits nuclear accumulation upon CITCO treatment that is delayed in COS-1 cells. Interestingly, CITCO induced nuclear accumulation of hCAR1. Mammalian two-hybrid and GST pull-down assays further demonstrated that hCAR1+A interacts with the co-activator SRC-1 and GRIP-1 at low level prior to activation, while at significantly enhanced level in the presence of CITCO. Thus, the alanine residue in the insertion of hCAR3 largely directs the xenobiotic response of hCAR3 through direct and indirect mechanisms. Activation of hCAR1+A may represent a sensitive methodology for the identification of hCAR activators.

PXR STATUS IS ASSOCIATED WITH CYP INDUCTION, HISTOPATHOLOGICAL EFFECTS, AND REDUCED CLEARANCE OF NONYLPHENOL.

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Nonylphenol, a byproduct of alkylphenol ethoxylates, is a ubiquitous chemical that binds to several nuclear receptors. Previous studies in our laboratory have demonstrated that nonylphenol also activates the pregnane X-receptor (PXR), a xenobiotic sensing nuclear receptor. Therefore, we are studying the role of PXR in protecting organisms from nonylphenol. Wild-type, PXR-null, and PXR×PXR-null mice with the human PXR receptor (mice) were treated with nonylphenol at 0, 50 and 75 mg/kg/day for one week and liver collected to determine nonylphenol concentrations. Wild-type mice treated with nonylphenol show induction of Cyp2b (males and females), as well as Cyp2c and Cyp3a (only in males). Induction of CYPs in wild-type mice was PXR-dependant as determined by the lack of induction in PXR-null mice. Interestingly, PXR mice only showed induction of Cyp2b. Liver histopathology indicated significant hepatocyte hypertrophy in nonylphenol-treated wild-type mice, indicating that wild-type mice had an acute response to compensate for nonylphenol exposure. PXR-null and PXR×PXR mice did not show any significant liver histopathological changes; however, these mice showed greater hepatocyte hypertrophy in untreated animals. Serum concentrations of nonylphenol were significantly higher in PXR-null mice compared to untreated mice. Taken together the histopathology, lack of CYP induction, and serum nonylphenol concentrations indicate that PXR-null mice are unable to respond to a nonylphenol challenge and eliminate the toxicant as observed in higher nonylphenol serum concentrations, and lack of CYP induction and liver hypertrophy. Overall this data suggest that PXR is important in eliminating nonylphenol and ultimately protecting individuals from its potential adverse effects. Funding for this research is provided by NIEHS.

CRITICAL ROLE OF Nrf2 CYSTEINE RESIDUES IN OXIDANT/ELETROPHILE-SENSING AND SIGNAL TRANSDUCTION.

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Cells respond to oxidants and electrophiles by activating receptor/transcription factors Nrf2 to coordinate induction of cytoprotective genes critical for defense against oxidative and other stresses. Activation involves blocking the ubiquitination-proteasomal degradation of Nrf2. Modification of cysteine thiol groups by oxidants in the linker region of Keap1, which congregates Nrf2 to the Keap1/Cul-3-based E3 complex for ubiquitination, is important but not sufficient for activation of Nrf2. Here we show that evolutionarily conserved cysteine residues of Nrf2 are critical for Nrf2 regulation. FLAH (a arsenic-based electrophile) and phenylarsine oxide (PAO) potently induce Nrf2 target genes and bind to Nrf2 in vitro and in vivo. Binding is inhibited by prototypical inducers arsine and rHBQ, PAO affinity pulldown and mutation of individual cysteine to alanine reveal that C235, C311, C316, C414 and C506 are critical for binding and binding is modulated by intra-molecular interactions. To corroborate the functions of cysteine residues, Nrf2 wild-type or mutants are expressed in Nrf2 knockout cells to reconstitute Nrf2 regulation. Nrf2 mutants have reduced 1/2 values that inversely correlate with increased binding to Keap1 and polyubiquitination of mutant proteins. Remarkably, the mutants fail to respond to arsine for Nrf2 activation and gene induction. Furthermore, mutations at C119, C235, and C506 impede binding of Nrf2 to endogenous antioxidant response element and to coactivator CBP/p300. The findings demonstrate for the first time that Nrf2 cysteine residues critically regulate oxidant/electrophile sensing, repress Keap1-dependent ubiquitination-proteasomal degradation, and promote recruitment of co-activators, such that chemical stimuli, receptor activation, and transcription activation are integrated at the receptor molecule.

SEARCHING FOR THE SPECIFIC INTRACELLULAR TARGET OF BORIC ACID THAT LEADS TO THE INHIBITION OF CALCIUM RELEASE IN PROSTATE CANCER CELL LINES.

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Boric acid (BA) is a dietary component found naturally in the environment. Low levels reduce the incidence and mortality of prostate cancer. Our current studies have shown that low doses of BA can inhibit calcium release from the ryanodine receptor (RyR) in the endoplasmic reticulum in response to RyR agonists in DU-145 prostate cancer cells. The dose of BA required to inhibit RyR-dependent release of stored calcium is 10 to 100 fold higher in non-tumor PWR-1E prostate cells than DU-145 cells, indicating that there may be differences in RyR isoform expression between cell lines. In this project our objective is to identify the specific RyR isoforms and/or accessory proteins that potentially interact with BA and cause the inhibition of calcium release. The identification of each cell line’s specific RyR isoforms was assessed with reverse-transcription PCR and restriction enzyme digestion. Both DU-145 and PWR-1E cells express RyR isoform 1 (RyR1) but LNCaP prostate cancer cells do not. All three cell lines express RyR2 and lack RyR3. Real-time PCR shows that RyR2 expression is lower in DU-145 cells than it is in the other two cell lines. Overall RyR mRNA expression in DU-145 cells treated with varying doses of BA was measured using real-time PCR. There was no significant change in mRNA expression indicating that BA exerts its effects at the receptor and not the transcription level. These results indicate that the BA-sensitivity between tumorgenic and non-tumorgenic cell lines is probably not due to a difference in RyR isoform expression. The cytoplasmic surface of all three RyR isoforms serves as a surface for binding of several accessory proteins that modulate opening and closing of the calcium channel. Future studies are needed to determine if differences in calcium release inhibition by BA can be explained by the interaction of BA with one or more of these accessory proteins or their RyR binding sites.

TRPV1 MEDIATES LUNG TOXICITIES OF SPECIFIC PARTICULATE MATERIALS.

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Particulate materials (PM) are ubiquitous air contaminants with established effects on human respiratory and cardiovascular health. Multiple features of PM have been correlated with adverse outcomes. However, the molecular sensors that detect, differentiate, and initiate responses to PM as well as the contributions of PM:PM sensor interactions to PM toxicity have not been fully elucidated. We hypothesized that specific calcium channels expressed by lung cells can both differentially detect PM and initiate responses that ultimately manifest as lung damage. Transient Receptor Potential Vanilloid-1 (TRPV1) over-expressing lung cells engineered to express high levels of cell surface TRPV1, were treated with multiple prototype PM. Calcium flux initiated by 3 of the PM, coal fly ash medium (CFAm), crystalline silica (Xtal), and synthetic residual oil fly ash (ROFA) was inhibited by the TRPV1
antagonists LJO-328 and capsaicin. Responses to CFAm and Xtal were also inhibited by EGTA and ruthenium red, while responses to ROFA were not, indicating selective activation of cell-surface TRPV1 by CFAm and Xtal. Responses to other PM were not attenuated by TRPV1 antagonists, but were blocked by EGTA and ruthenium red, demonstrating that additional cell surface calcium channels are PM sensors. CFAm leached with aqueous and organic solvents retained full agonist activity and activation was governed largely by the acid-sensing domain of TRPV1 (E64B), not the intra-membrane vanilloid binding site (Y511). Intracellular instillation of capsaicin (0.5 mg/kg), CFAm or Xtal (0.25 mg) into mouse lungs increased expression of IL-6 and TNFα and caused inflammation after 4h. These outcomes were attenuated in TRPV1-deficient mice. Conversely, responses to CFAhigh, a PM that caused calcium flux independent of TRPV1, were not attenuated. These data indicate that TRPV1 is a specific mediator of acute lung toxicity elicited by some forms of PM and highlights the value of the TRPV1 over-expressing cell system for identifying and characterizing molecular sensors of PM. Support: HL069813 and ES017431.

2152 ELUCIDATING THE CONSTITUTIVE AND INDUCIBLE ACTIVITY OF THE HUMAN TNIP1 PROMOTER.


Nuclear receptors are ligand-dependent transcription factors whose ability to modulate transcription of target genes is determined by the receptors’ ligands and a class of special proteins known as coregulators. Our laboratory has isolated a novel nuclear receptor coregulator, TNPAP1 interacting protein 1 (TNP1P1) in a yeast two-hybrid screen and characterized it as a corepressor of retinoic acid receptors (RARs) and peroxisome proliferator-activated receptors (PPARs). RARs and PPARs are pharmacologic targets for control of various pathologies including but not limited to: diabetes, psoriasis, photoaging, and various cancers. As TNIP1 represses activity of RARs and PPARs, it becomes crucial to understand what controls its expression. We have isolated ~6kb of the human TNIP1 promoter and examined it in silico and experimentally for transcriptional control elements. Sequence analysis by MatInspector predicted two specificity protein (Sp) sites in the proximal region of the promoter and three NF-kB sites in both the proximal and distal regions of the TNIP1 promoter. Chromatin immunoprecipitation demonstrated the physical association between specific regions of TNIP1 promoter and Sp1, Sp3, and NF-kB. EMSA showed Sp1 and NF-kB binding at distinct sites in both the proximal and distal regions of the TNIP1 promoter. We hypothesized the Sp family of transcription factors and NF-kB would contribute to TNIP1 promoter constitutive and inducible activity, respectively. Transcription activation studies revealed TNIP1 is positively regulated by Sp1, Sp3, and NF-kB. Furthermore, EMSA showed Sp1 and NF-kB binding at distinct sites in both the proximal and distal regions of the TNIP1 promoter. In summary we have demonstrated that Sp1, Sp3, and NF-kB contribute to constitutive and inducible regulation of the TNIP1 promoter through proximal and distal sites. Changes in endogenous NF-kB or pharmacological control of its activity would be expected to affect TNIP1 expression beyond that driven by Sp1 which, in turn, could ultimately regulate RAR and PPAR activity.

2153 CONTRIBUTIONS OF TRPV1, THE ENDOPLASMIC RETICULUM STRESS RESPONSE, AND ENDOVANILLOIDS TO INFLAMMATORY LUNG INJURY AND LUNG CELL DEATH IN VITRO.

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Endogenous TRPV1 agonists are implicated in lung inflammation and injury during sepsis and severe trauma such as burn injury. Activation of TRPV1 in cultured human lung cells by agonists disrupts calcium homeostasis, activates the ER stress response, and causes cell death. Similarly, treatment of mice with LPS (i.p.) or capsaicin (i.t.) produces ER stress, lung inflammation, and injury. ER stress, assessed by GADD153 expression and other pathologic outcomes were attenuated by TRPV1 antagonist LJO-328 and in mice lacking the TRPV1 gene. Here we further investigated the relationship between TRPV1, ER stress, and sensitivity of lung cells to the endogenous TRPV1 agonist anandamide in lung cell types that are targets of damage during systemic inflammation. TRPV1 expression was quantified by qRT-PCR in primary normal human bronchial epithelial cells and human lung microvascular endothelial cells. The relative level of TRPV1 mRNA expression correlated with ER stress/GADD153 expression and cell death following treatment with the prototype TRPV1 agonist nonivamide. Similarly, treatment with the endogenous TRPV1 and cannabinoid receptor (CB1/2) agonist anandamide caused ER stress/GADD153 induction and cell death, but neither LJO-328 nor the CB1/2 antagonist AM-251 were inhibitory. Additionally, cell death caused by anandamide did not correlate with TRPV1 mRNA expression, also indicating a TRPV1- and CB1/2-independent mechanism of ER stress and cytotoxicity for this endovaniloid. These results indicate that activation TRPV1 by prototypical and endogenous TRPV1 agonists causes ER stress in lung cells damaged during severe systemic inflammation and in the intact lung. Activation of TRPV1 appears to be an integral contributor to lung inflammation and injury. However, anandamide is probably not a major activator of TRPV1-dependent phenomena in our model systems. Support: NIH grant HL069813 and the Univ. of Utah Dept. of Anesthesiology.

2154 CAR AND PXR-DEPENDENT TRANSCRIPTIONAL CHANGES IN THE MOUSE LIVER AFTER EXPOSURE TO THE FUNGICIDE AND MOUSE LIVER CARCINOGEN, PROPICONAZOLE.

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Exposure to the conazole, propiconazole causes tumors in mice after 2 years. Previous transcript profiling of livers from wild-type mice after short-term exposure to conazoles revealed signatures indicative of CAR and PXR nuclear receptor signaling. To test the hypothesis that CAR and PXR mediate many of the transcriptional changes, wild-type, CAR-null and PXR-null mice were administered vehicle or propiconazole (210 mg/kg/day) by gavage for 7 days and gene expression was determined by Affymetrix full-genome gene chips. PXR and CAR genotype had profound effects on the hepatic transcript profiles. About 85% of all the genes regulated by propiconazole in wild-type mice were dependent on PXR and included cholesterol biosynthesis genes. The behavior of the remaining ~15% of the genes significantly overlapped with those regulated by the CAR activators, phenobarbital or TCPOBOF; in wild-type, but not CAR-null mouse livers. Transcript profiling of the liver from wild-type mice revealed that ~79% of all genes regulated by propiconazole were dependent on CAR. The CAR-independent genes included many xenobiotic metabolizing genes known to be regulated by PXR. CAR-null and PXR-null mice exhibited higher serum cholesterol levels than wild-type mice, indicating the role of these nuclear receptors in cholesterol homeostasis. Overall, these results indicate the importance of both CAR and PXR in the transcriptional effects of propiconazole. (This abstract does not necessarily reflect U.S. EPA policy).

2155 CHARACTERIZATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA (PPARALPHA) – INDEPENDENT EFFECTS OF PPARALPHA ACTIVATORS IN THE RODENT LIVER: DI-(2-ETHYLHEXYL) PHTHALATE ALSO ACTIVATES THE CONSTITUTIVE ACTIVATED RECEPTOR.

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Peroxisome proliferator chemicals (PPC) are thought to mediate their effects in rodents on hepatocyte growth and liver cancer through the nuclear receptor peroxisome-proliferator-activated receptor alpha (PPARα). Recent studies indicate that one such PPC, the plasticizer di-2-ethylhexyl phthalate (DEHP), increases the incidence of liver tumors in PPARα-null mice. We hypothesized that some PPC, including DEHP, induce transcriptional changes that are independent of PPARα but dependent on other nuclear receptors, including the constitutive activated receptor (CAR). CAR is known to mediate the effects of phenobarbital (PB) on hepatocyte growth and liver tumor induction. To determine the potential role of CAR in mediating the effects of PPC, a meta-analysis was performed in which the transcript profiles from published studies using PPC-exposed rats and mice were compared to those produced by exposure to PB, Valproic acid, clofibrate and DEHP in rat liver and DEHP in mouse liver induced genes known to be regulated by CAR, including Cyp2b family members. Further examination of transcript changes using Affymetrix Mouse Gene 1.0 ST arrays and RT-PCR in the livers of DEHP-treated wild-type, PPARα-null and CAR-null mice demonstrated that 1) most (94%) of the transcriptional changes induced by DEHP were PPARα-dependent, 2) many PPARα-independent genes overlapped with those regulated by PB, 3) induction of Cyp2b10, Cyp3a11 and metallothionein-1 by DEHP was CAR-dependent but PPARα-independent and 4) induction of a number of genes (Cyp2b11, Gstm4,
Gstm7 was independent of both CAR and PPARα. Our results indicate that exposure to activators of PPARα, including DEHP, could activate multiple nuclear receptors in the rodent liver. (This abstract does not necessarily reflect U.S. EPA policy).

2156  LIVER MRNA AND MRNA PROFILING OF PHENOBARBITAL (PB)-TREATED PXR/CAR DOUBLE KNOCK-OUT AND HUMANIZED MOUSE MODELS PROVIDES INSIGHT INTO MECHANISM(S) OF PB-MEDIATED MOUSE HEPATOCARCINOGENESIS.
CAR Biosciences Ltd., Dundee, United Kingdom and TaciteAriens GmbH, Cologne, Germany.

We have created mouse lines (C57BL/6j) background) in which the murine pregnane X receptor (PXR) and constitutive androstane receptor (CAR) genes have been simultaneously removed (PXR KO/CAR KO mice) and/or replaced with human PXR and CAR (hPXR/hCAR mice). Previously, PB-treatment increased hepatic gene expression in wild type (WT) mice but not in hPXR/hCAR mice. Ten PXR KO/CAR mice with humanized PXR and CAR were created by replacing the murine genes with human genes and then crossing them with WT mice. We also crossed human PXR KO/CAR mice with mice expressing human CAR (hCAR mice). We have analyzed these mice for the expression of a number of genes regulated by PXR and CAR.

2157  LOSS OF PREGNANE X RECEPTOR CONTRIBUTES TO COLON CANCER CELL INVASION VIA REGULATING β-CATENIN CYTOPLASMIC ACCUMULATION.
Texas A&M University, College Station, TX.

The accumulation of β-catenin in cytoplasm plays a critical role in the adhesion and invasion of cancer cell. Pregnan X receptor (PXR), which was originally identified as a gene that regulates metabolism of xenobiotics and endobiotics, was reported to play a novel function on promoting β-catenin degradation. Methods: the relationship between the expression of β-catenin and PXR expression in normal colon mucosa and colon cancer was investigated by immunohistochemical staining in APC Min/+ mice. The human colon cancer cell line HT-29 cell which has APC mutation were stablely transfected with PXR gene, assayed for migration and invasion of cancer cell. Pregnane X receptor (PXR), which was originally identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identified as a gene that regulates metabolism of xenobiotics and endobiotics, was re-identifi-
single exposure. Colony formation and MTT colorimetric assays revealed a dose-dependent decrease in cellular viability in coexposed cells. Western blot analysis for cells coexposed to NiCl2 and CoCl2 exhibited increased PARP cleavage along with decreased expression of MLH1 and MSH2, two proteins involved in mismatch repair (MMR). This suggests that interference with MMR may be a mechanism by which these metals elicit their response. The increase in apoptosis in coexposed cells did not alter protein levels of p53. Lastly, coexposure resulted in a dose-dependent increase in the formation of micronuclei. Altogether, the findings of this study demonstrate that coexposure to heavy metals elicits greater adverse toxicological effects than single exposure resulting in enhanced cytotoxicity and genotoxicity.

2164 TRANSFORMATION-DISSOLUTION STUDIES OF TUNGSTEN SUBSTANCES. R. Lemus1, K. Heim1, J. Skoelf1, M. Jackson1 and M. Pardus1. 1ARCADIS U.S. Inc., Pittsburgh, PA and 2CANMET-FMMTL, Ottawa, ON, Canada.

According to the United Nations Globally Harmonized System for Classification and Labeling of Chemicals (UN GHS) and the European Union classification system, the evaluation of the short-term and long-term aquatic toxicity of metals and sparingly soluble metal compounds is conducted by comparison of the concentration of the relevant metal ion in solution, with appropriate ecotoxicity reference values (acute and chronic) derived from the toxicity of the relevant soluble metal salt. The amount of soluble metal ion was determined by transformation/dissolution (T/D) testing of the metal substance, as described in Annex 10 of the UN GHS and the draft OECD test guideline. T/D studies tested ammonia paratungstate (APT), ammonium metatungstate (AMT), sodium tungstate (Na2WO4), tungsten trioxide (WO3), tungsten blue oxide (TBO), tungsten carbide (WC), and tungsten metal powder (W) in the standard aqueous media, which are representative of conditions found in the environment. The tests were conducted by stirring standard quantities (1, 10, or 100 mg/L) of the tungsten test substance at pH 6 or 8.5 in the standardized aqueous medium which contained NaHCO3, KCl, CaCl2, and MgSO4. The concentrations of dissolved tungsten (W) were measured (using ICP-MS) at standard time intervals of 24 hours (screening test), and at intervals of 7 and 28 days for less soluble substances (full test). The dominant metal ion species of the dissolved W was also measured (using HPLC) and was confirmed to be tungstate. For all substances tested at both pHs, higher W and tungstate concentrations were obtained at pH 8.5 than at 6. Comparing the results obtained for each substance demonstrated that the amount of dissolved W and tungstate ion is largely determined by the water solubility of the substance and the extent to which it can react with the media to transform to water-soluble forms of the relevant metal. The results demonstrated three levels of release of dissolved W-species: (1) low for W and WC; (2) medium for WO3 and TBO; and (3) high for Na2WO4, AMT, and APT.

2165 BIOACCESSIBILITY STUDY OF HARDMETALS. K. E. Heim1, M. Jackson1, R. Lemus1, D. Capellini2 and M. Pardus1. 1ARCADIS, Durham, NC and 2Kirby Memorial Health Center, Wilkes-Barre, PA.

Occupational exposure to some types of hardmetal has been associated with hardmetal disease, a condition that causes a type of pneumonitis. Hardmetal constitutes a broad category of composite materials that are made up of tungsten carbide (WC) plus cobalt (Co) and may include a variety of other metals, and are employed for a variety of uses. Occupational exposure to hardmetals may be to the pre-sintered or post-sintered hardmetals, whereas consumer exposure is only to post-sintered hardmetals. Standard testing of metal compounds in vitro, such as a surrogate for bioaccessibility has been used to facilitate hazard and risk assessment. The potential to release Co ions was tested using in vitro bioaccessibility testing on Co powder and two hardmetal containing approximately 6% Co and 94% WC (one pre-sintered and one post-sintered). Bioaccessibility testing in simulated human lysosomal fluid (simulating respiratory exposure) measured the amount of Co ion release. The methodology for testing followed that described in the European Union Draft Guidance for the RIP (REACH Implementation Plan) 3.6: Bioavailability and Read-Across for Metals and Minerals. Extractions were performed in triplicate using 0.1 g of sample in 50 ml of simulated human lysosomal fluid at pH 4.5, 2.5, 2.4, and 72 hours. At 2 hours, the amount of Co released averaged 1.1 g Co/g Co powder, 0.62 g Co/g post-sintered hardmetal, and 0.44 g Co/g pre-sintered powder. The 5 hour time point Co ion release values for pre- and post-sintered hardmetal were similar (0.61 g Co/g and 0.60 g Co/g, respectively), and continued to be similar for the remaining time points. These results show that a significant amount of Co in the hardmetals was released into solution, with sintering slightly delaying the cobalt ion release. Further testing is ongoing for 12 other hardmetals in lysosomal and other simulated human fluids, measuring the release of the major constituent metal ions. These data will help assess whether the exposure to different hardmetals could result in human health effects.

2166 BIOAVAILABILITY STUDY OF FIVE TUNGSTEN SUBSTANCES USING SIMULATED GASTRIC, ALVEOLAR, INTERSTITIAL, LYSOSONAL, AND SWEAT FLUIDS. M. Jackson1, K. Heim1, R. Lemus1, M. Muzzio1 and M. Pardus1. 1ARCADIS U.S. Inc., Pittsburgh, PA and 2Left Sciences Group, IIT Research Institute, Chicago, IL.

Testing of metal compounds for solubility in synthetic fluids in vitro (bioaccessibility) as a surrogate for in vivo bioavailability testing has been used to facilitate hazard and risk assessment. Therefore, the aim of this study was to measure the bioaccessibility...
bility of tungsten trioxide (WO3), tungsten blue oxide (TBO), fused tungsten carbide (WC), tungsten metal powder (W), and tungsten carbide (WC) in simulated human gastric (GF), sweat (SF), alveolar (AF), lysosomal (LF), and interstitial fluids (IF). Extractions were performed in triplicate using 0.1 g of sample in 50 ml of fluid, at 37 °C. Simulated GF was sampled after 5 hours; simulated IF, AL, and LF were sampled after 2, 5, 24, and 72 hours; and simulated SF was sampled after 12 hours. The fluid samples from all time points were analyzed for dissolved tungsten by ICP-MS. The percent of available tungsten for WO3, TBO, WC, W, and WC was 0.0057, 0.08, 0.34, 0.25, and 0.0357% respectively (GF); 8.2, 1.9, 0.9, and 0.081% respectively (SF); 40, 42, 0.64, 0.79, and 0.14% respectively (IF); 35, 43, 3.4, 1.1, and 0.52% respectively (AF); and WO3, TBO, WC, W, and WC was 39, 63, 4.6, 1.1, and 0.94% respectively (LF). These results do not allow for quantification of the amount of the substances that would be absorbed, but instead provide a relative potential for absorption. The great variability of tungsten's bioaccessibility was observed at 72 hours in the LF, IF, and AF environment. Bioaccessibility data from testing in GF and SF indicate that these five tungsten substances will not be significantly absorbed as a result of oral or dermal exposure. Based on the AE, LF, and IF results, it appears that WO3 and TBO are more likely to relate tungsten ions and be absorbed by the human body than WC, WC, W, and WC as result of inhalation exposure.

**2167** REGULATION OF CYTOCHROME P450 IA1 (CYP1A1) BY VANADIUM IN HUMAN HEPATOMA HEPG2 CELLS.

G. Abdelhamid, A. Anwar-Mohamed and A. O. El-Kadi, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

We recently demonstrated that V5+ down-regulates 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-mediated induction of Cyp1a1 mRNA, protein, and catalytic activity levels in Hepa 1c1c7 cells through transcriptional mechanism. Therefore, it is important to investigate whether similar changes occur in humans. For this purpose, we used 0.0057, 0.08, 0.34, 0.25, and 0.0357% respectively (GF); 8.2, 1.9, 0.9, and 0.081% respectively (SF); 40, 42, 0.64, 0.79, and 0.14% respectively (IF); 35, 43, 3.4, 1.1, and 0.52% respectively (AF); and WO3, TBO, WC, W, and WC was 39, 63, 4.6, 1.1, and 0.94% respectively (LF). These results do not allow for quantification of the amount of the substances that would be absorbed, but instead provide a relative potential for absorption. The great variability of tungsten's bioaccessibility was observed at 72 hours in the LF, IF, and AF environment. Bioaccessibility data from testing in GF and SF indicate that these five tungsten substances will not be significantly absorbed as a result of oral or dermal exposure. Based on the AE, LF, and IF results, it appears that WO3 and TBO are more likely to relate tungsten ions and be absorbed by the human body than WC, WC, W, and WC as result of inhalation exposure.

**2168** ACTIVATION OF JAK STAT IN MEGAKARYOCYTES BY INHALED VANADIUM.

A. Gonzalez-Villalva, V. Rodriguez-Lara and T. L. Fortoul, Biologia Celular y Tisular, Facultad de Medicina, UNAM, Mexico City, Mexico.

Vanadium as part of inhaled suspended particles enters the circulatory system and induces changes in a variety of systems. Our previous work had demonstrated spleen thrombocytopathy and megakaryocytosis after its inhalation. We explore the activation of the JAK/STAT signaling in megakaryocytes by cytofluorometry in order to explain the blood changes observed. CD male mice were exposed to V2O5 [0.02M] twice a week for 8 weeks. An increase in phosphorylated JAK2 and STAT3 was observed at the end of the exposure. The activation of these proteins suggests that the activation of this signaling pathway is related with increase of production of tumor necrosis factor alpha (TNF-alpha) and its function as macrophage and thrombocytopathy.

Supported by DGAPA-UNAM PAPIIT IN210409, CONACyT scholarship recipient

**2169** ACTIN CHANGES IN TESTICULAR CELLS AFTER VANADIUM PENTOXIDE INHALATION.

V. Rodriíguez-Lara, A. Morales-Rivero and T. L. Fortoul, Biologia Celular y Tisular, UNAM, Mexico City, Mexico.

Vanadium is a transition metal emitted to the atmosphere during the combustion of fossil fuels. Reprotoxic effects of vanadium like low sperm count, decreased sperm motility, and sperm morphologic abnormalities had been reported. These alterations are proposed as direct consequences of the interactions of vanadium with cytoskeletal proteins; however no information is available relating vanadium and actin. We investigated the alterations in actin of testicular cells after vanadium pentoxide inhalation. CD-1 male mice were exposed twice a week for six weeks to vanadium pentoxide [0.02M]. Mice were sacrificed at 1, 3 and 6 weeks: testes were removed, fixed and processed for standard immunohistochemistry. Control and exposed mice were analyzed and quantified with image J program. A percentage of immunoreactive cells to actin, was calculated in controls and exposed mice. The results indicated that vanadium exposure produce a time dependent decrease in percentage of immunoreactivity actin testicular cells. Our results explain the reproductive effects of vanadium due to actin alterations produces changes in hematotesticular barrier permeability and spermatogenesis changes related with infertility.

**2170** STUDY ON MECHANISM FOR DEPLETED URANIUM-INDUCED TRANSFORMATION IN HUMAN LUNG EPITHELIAL CELLS.

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Depleted uranium (DU) is commonly used in military applications and is also used in civilian industry and thus exposure of soldiers and others is frequent and widespread. There is limited research information on the potential health hazards of DU exposure. In our study we used the human bronchial epithelial cell line (BE2PD) to study the potential carcinogenic hazard of DU. We observed that cells arrested in S phase in a concentration-dependent manner after 24 h exposure. We found that DU induces cell transformation including cell contact inhibition and anchorage independent phenotype. Doses of 0.25, 2.5 and 25 μg/cm2 uranium trioxide induces 23, 24 and 17 grow foci in 30 dishes, respectively. These foci were cloned and found to exhibit anchorage independent and an aneuploid phenotype. We next investigated DU-induced epigenetic modifications. We found that DU decreases trimethylated H3K9 (H3K9me3) which is a marker of DNA methylation, along with inhibition of the DNA damage repair protein ATM. Our study demonstrates that in addition to genotoxicity, DU-induced aberrant methylation may also contribute to its carcinogenesis. This work was supported by ARO grants # W911NF-04-1-0240 and W911NF-08-0033 (J.PW).

**2171** DEPLETED URANIUM INDUCED DNA SINGLE STRAND BREAKS IN CHINESE HAMSTER OVARY CELLS.

M. Yellowit¹ and R. Lanzu², ¹Pharmacology & Toxicology, The University of Arizona, Tucson, AZ and ²Cell Biology and Anatomy, The University of Arizona, Tucson, AZ.

DNA damage has been implicated in the genotoxicity of many heavy metals. The aim of this study is to evaluate the genotoxicity of depleted uranium (DU) in Chinese Hamster Ovary cells (CHO) with mutations in various DNA repair pathways. CHO cells were exposed to 0.003–300 μM of soluble depleted uranium as uranyl acetate (UA) for 0–48 hr. Intracellular DU concentrations were measured via inductively coupled mass spectrometry (ICP-MS). Cytotoxicity was assessed in vitro by clonogenic survival assay. DNA damage response was assessed via Fast Micromethod® to determine UA-induced DNA single strand breaks. Results indicate that UA is entering the CHO cells, with the highest amounts found in the nucleus of all cell lines compared to the cytosol. Clonogenic assay shows that UA is cytotoxic in each cell line with the greatest cytotoxicity in the base excision repair deficient EM9 cell line and the nuclear excision repair deficient UV5 cell line compared to the non-homologous end joining deficient V3.3 cell line and the parental AA8 cell line after 48 hr. This may indicate that UA is producing single strand breaks and forming UA-DNA adducts rather than double strand breaks in CHO cells. Fast Micromethod® results indicate an increased amount of single strand breaks in the EM9 cell line after 48 hr UA exposure compared to the V3.3 and AA8 cell lines. These results are consistent with previous studies that indicate DU induces DNA damage via strand breaks and uranium-DNA adducts in treated cells. These results suggest that: (1) DU is genotoxic in CHO cells, and (2) DU maybe inducing single strand breaks rather than double strand breaks in vitro. We are currently examining the possible formation of UA-DNA adduct formation to better understand the DNA damage stimulated by exposure to DU. This work is supported by NIH Grants CA096281 (RCL), F31ES014971 (MY), P30ES006694, 23, 24 and 17 growth foci in 30 dishes, respectively. These foci were cloned and found to exhibit anchorage independent and an aneuploid phenotype. We next investigated DU-induced epigenetic modifications. We found that DU decreases trimethylated H3K9 (H3K9me3) which is a marker of DNA methylation, along with inhibition of the DNA damage repair protein ATM. Our study demonstrates that in addition to genotoxicity, DU-induced aberrant methylation may also contribute to its carcinogenesis. This work was supported by ARO grants # W911NF-04-1-0240 and W911NF-08-0033 (J.PW).
EVALUATION OF ZINC BIOAVAILABILITY TO THE STRATOM CORNEUM FROM A BISGLYCINATE CHELATE IN VITRO.

J. W. Hartle1 and L. B. Joseph.1 1ReD, Albion, Clearfield, UT and 2Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.

Zinc is a trace element essential for the survival and function of all cells. Oral supplements containing a zinc bisglycinate chelate have a higher zinc bioavailability than zinc oxide. Minerals are currently used in many cosmetic and wound therapy topical preparations, where a major hurdle to efficacy is penetration of the stratum corneum. In these studies, the absorption of zinc from zinc bisglycinate chelate versus zinc oxide into the skin was investigated. To evaluate whether a zinc chelate is absorbed into the skin better than an inorganic form, zinc bisglycinate chelate and zinc oxide were compared in non-ion, oil in water and water in oil topical preparations in an in vitro skin model (EpidermTM). Test formulations were weighed directly onto the tissue and the tissue was placed on a Franz cell with a 5 mL reservoir filled with phosphate buffered saline. After 8 hours of exposure, the tissue was removed from the Franz cell and any of the remaining topical preparation was analyzed for residual zinc. The reservoir fluids, wash solutions, and tissues were analyzed for zinc content. It was determined that zinc bioavailability from either zinc source was independent of the topical preparation. The amount of zinc absorbed into the EpidermTM from the zinc bisglycinate chelate was 6.54% from a non-ionic formulation, 6.58% from an oil in water formulation and 7.15% from a water in oil formulation, as compared to 1.61%, 2.86% and 1.95% respectively for ZnO (p<0.05). These data suggest that zinc, in the form of zinc bisglycinate chelate was more readily absorbed from a topical preparation as compared to zinc oxide. Therefore, zinc from a zinc bisglycinate chelate, a known oral supplement, has superior bioavailability in the stratum corneum as compared to zinc oxide.

ULTRASTRUCTURAL AND TOXICOPROTEOMIC STUDIES OF STRUCTURE AND FUNCTION OF THE BLOOD-CSF BARRIER IN MANGANESE-EXPOSED RAT MODEL.

G. J. Li1,2, H. M. Jing1, K. H. Wei1, F. Yang3, W. H. Gao1, C. Y. Zhao1, L. Ma1, J. Z. Liu1, T. Zhang1 and W. Zheng1. 1Research Center for Preventive Medicine, Beijing, China, 2Capital Medical University, Beijing, China, 3Beijing Proteome Research Center, Beijing, China and 4Purdue University, West Lafayette, IN.

Toxicoproteomics is a newly evolved discipline in toxicological research. This study was designed to test if chronic Mn exposure led to changes in protein expression and structural damage in the choroid plexus (CP), a brain tissue critical to material exchanges in the body. CP tissues were collected at each time point. Light microscopy and TEM revealed that Mn exposure was ceased. A 2-DE coupled with nanoLC-Q-TOF MS and differential proteomics analysis showed that among 168 BCB proteins identified, about 20 proteins in the CP were up- or down-regulated following Mn treatment. Further verification and analysis are in progress. Taken together, these data suggest that Mn in the CP not only damages the structural integrity, but also alters the expression of proteins critical to control of the blood-CSF barrier. Further studies are needed to determine whether Mn exposure has any impact on the CP structure and function. Further verification and analysis are in progress. Taken together, these data suggest that Mn in the CP not only damages the structural integrity, but also alters the expression of proteins critical to control of the blood-CSF barrier. Further studies are needed to determine whether Mn exposure has any impact on the CP structure and function.

TELLURIUM TETRACHLORIDE INDUCES APOPTOSIS IN RAT HIPPOCAMPAL ASTROCYTES.

D. Harde1 and S. Roy. Pharmacological Sciences, St. John’s University, Jamaica, NY.

Tellurium (Te), a metalloid, is increasingly being used in industry as a component of semiconductor factors and in the production of optical magnetic disks. Environmental exposure and accumulation of Te by plants will increase human exposure. Diphenyl ditelluride has been shown to cause peripheral demyelination in rats. This effect is attributed to inhibition of squalene epoxidase, an enzyme necessary for cholesterol biosynthesis. Reported effects of tellurium compounds on the central nervous sys-
used by real-time PCR array and release of IL-8 and IL-6 was measured by ELISA. The PCR array showed that Zn induced different cytokine pattern than Fe, with high mRNA levels of CCL11, CCL26, CXCL5 and CXCL14. Both metals had high expression of CXCL8 and CCL20. The metals Cd, As and Zn were the most potent to induce the release of CXCL8 and IL-6, followed by Mn, Ni and V, and the least potent metals were Cu and Fe. The metals showed also differential effects in induction of apoptosis as well as necrosis in the epithelial lung cells. In conclusion, metals showed marked differences with regard to inflammatory and cytotoxic properties in BEAS-2B cells. This may indicate that metals play a role in the inflammatory processes induced by particulate matter, and that the metals’ contribution vary depending on types of sources.

Both civilians and military personnel can be exposed to toxic chemicals and materials from occupational sources, environmental pollution, or as the result of military activity. The goal in this first stage of our study was to measure oxidative stress markers after different transition metal exposures. We performed a toxicological study to examine these effects using rats treated through I.P. injection. Sprague-Dawley rats were dosed with NiCl2 (0.25, 0.5, 0.75 mmol/kg BW), Na2Cr2O7 (5, 10, 20 mg/kg BW) and CdCl2 (0.5, 1.25, 2.5 mg/kg BW) and humansly sacrificed 1, 3 and 7 days post exposure. Liver tissue, kidney tissue, blood and lung lavage fluid were collected and analyzed. Liver tissue showed an increased oxidative damage, through lipid peroxidation and hydrogen peroxide production, in all metal exposures with Cr being the most dramatic and Ni showing damage at days 3 and 7. Kidney tissue demonstrated immediate damage from Cr and then showed recovery, while Cd and Ni showed effects at day 7, at the highest concentration. Electron spin resonance results showed an increase in hydroxyl radical formation in liver and kidney tissue from Cr, day 1, as well as Cd & Ni on days 3 and 7, at the highest exposure levels. Bronchopulmonary lavage was also performed on the rats to yield macrophages and cell differential measurements. This dramatic rise in Cr exposed animals indicated a cross talk from the I.P. exposure. To summarize, Cr-induced oxidative damage at day 1 was reduced or resolved at day 7, while Cd and Ni produced more oxidative damage at days 3 and 7 at the higher exposure levels. This data will be combined with the second stage of our study which involved the analysis of blood biomarkers and gene transcripts to develop a method of identifying early biomarkers of transition metal exposure.

Adverse cardiovascular effects have been shown following particulate matter pulmonary exposure. In this study we evaluated acute systemic inflammation following aspiration exposure to three different types of welding fume (manual metal arc-stainless steel [MMA-SS]; gas metal arc-SS [GMA-SS]; GMA-mild steel [GMA-MS]). Fumes generated from SS electrodes are approximately 40-50% iron, stainless steel [MMA-SS]; gas metal arc-SS [GMA-SS]; GMA-mild steel [GMA-MS]). Fumes generated from SS electrodes are approximately 40-50% iron, stainless steel [MMA-SS]; gas metal arc-SS [GMA-SS]; GMA-mild steel [GMA-MS]). Fumes generated from SS electrodes are approximately 40-50% iron, stainless steel [MMA-SS]; gas metal arc-SS [GMA-SS]; GMA-mild steel [GMA-MS]). Fumes generated from SS electrodes are approximately 40-50% iron, stainless steel [MMA-SS]; gas metal arc-SS [GMA-SS]; GMA-mild steel [GMA-MS]).

Results of epidemiological studies indicate that emergency room visits for respiratory indications increase during periods of Florida Red Tides. The purpose of this study was to examine whether repeated daily brevetoxin inhalation, as may occur during a Red Tide, alters viral clearance, and pulmonary responses to influenza A. Male F344 rats were divided into four groups: 1) sham aerosol/ no influenza; 2) sham aerosol/ influenza; 3) brevetoxin/no influenza; and 4) brevetoxin/ influenza. Animals were exposed by nose-only inhalation to vehicle or 50 μg brevetoxin/mL, 2 hr/day for 12 days. On the sixth day of aerosol exposure, Groups 2 and 4 were administered 10,000 plaque forming units of non-adapted influenza A, strain HKX-31 (H3N2). Subgroups were euthanized 2, 4, and 7 days post influenza treatment. Left lungs were taken for histopathologic evaluation and right lungs evaluated for viral load and cytokine content. Influenza virus was cleared from the lungs over the 7 day period, however, there was significantly more virus (1.4 times) remaining in the Group 4 lungs compared to Group 2 lungs. At 7 days following influenza instillation, the severity scores for perivascular and peribronchial infiltrates were higher in Group 4 compared to Group 2, however, the severity score for alveolar macrophage hyperplasia in Group 4 was approximately half that in Group 2. The severity scores for bronchiolitis were approximately equal in Groups 2 and 4 at 48 hours post influenza treatment. Bronchiolitis persisted, with low incidence and severity, only in Group 4 at 4 and 7 days. Influenza significantly increased interleukins 1-α and 6 and monocyte chemotactic protein-1 in lung, compared to Group 1 rats; brevetoxin exposure significantly increased the influenza – induced responses. These results suggest that repeated inhalation exposure to brevetoxin may slightly impair clearance and enhance the pathogenicity of influenza A in the rat lung. Research conducted under NIEHS P01 ES10594.

There is a growing concern regarding the health impact of various nanoparticles. The aim of the present study was to compare the allergy-promoting capacity of carbon nanofibers (CNF) and nanotubes (CNT). We also aimed to identify major physicochemical characteristics important for the biological effects of CNF and CNT. Four qualitatively different CNF samples from one manufacturer, as well as single-walled (sw) and multi-walled (mw) CNT, were tested in two allergy mouse models. The particles were given by s.c. injection into the footpad or intranasally to BALB/c mice together with the allergen ovalbumin (OVA). After an allergen booster, the allergic response was determined by measuring OVA-specific IgE in serum and eosinophil numbers in the bronchoalveolar fluid (BALF). OVA-specific IgG1 and IgG2a in serum and inflammatory cells and mediators (MCP-1 and TNF-α) in BALF were also measured. The four CNF samples and the sw and mw CNT all increased the OVA-specific IgE levels. The IgE response was markedly stronger for the sw and mw CNT, which also was associated with an eosinophil inflammation in the lung. The four CNF samples with different physicochemical characteristics had similar IgE adjuvant capacity in the airways. However, two of the CNF samples with several characteristics in common stood out from the other CNF with regard to a number of the other endpoints. Overall, we demonstrate that CNF and CNT promote allergic responses in two allergy models in mice, and that nanofibers have specific properties to be exploited for allergic adjuvant effects. Further, our data suggest that particle properties like the tube structure, fiber or tube width, relative surface area, metal content and structural defect sites all deserve attention in future toxicological studies, to enable optimization of the production process towards less toxic nanoparticles.
2181 EVALUATION OF INNATE AND HUMORAL IMMUNITY FOLLOWING IN VIVO EXPOSURE TO MICRO AND NANOFIBROUS ELECTROSPUN POLYCAPROLACTONE FOR TISSUE ENGINEERING APPLICATIONS.

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Electrospun materials are being widely investigated for potential use in many soft tissue applications, such as polycaprolactone (PCL) for a vascular graft material. Electrospinning allows for the manipulation of various material properties including fiber diameter, which can range from the micron to the nanometer scale. Previously, there have not been in vivo evaluations of the potential immunotoxicity of electrospun polymers however in vitro work has shown various electrospun polymers, including PCL, may be immunosuppressive. We are currently evaluating the potential immunomodulation following exposure to micro vs. nanofibrous electrospun PCL implanted subcutaneously in young adult vs. aged (> 6 months old) B6C3F1 mice. Implants of micro and nanofibrous electrospun PCL are implanted in the ventral quadrant in doses of one, two, or four 16mm2 disks. Assays are conducted on day 29 following 28 days of material exposure. The ultimate goals of this work are to assess effects on innate, humoral, and cell-mediated immunity. Effects on innate immunity have been assessed using the natural killer cell activity assay, which has shown no effect with both microfibrous and nanofibrous PCL. The sheep red blood cell (sRBC) IgM ELISA and sRBC antibody forming cell (AFC) assay have been used to evaluate humoral immunity. The sRBC ELISA has shown no effect with both microfibrous and nanofibrous PCL, while the AFC assay has shown some statistical differences between controls and material in young animals none have been dose dependent. An initial study with aged animals at the time of implantation indicates that microfibrous PCL may have dose-dependent suppressive effects on humoral immunity in aged animals. Current studies indicate that while electrospun polycaprolactone may have minimal to no effect on the immune system of young animals it may cause immunosuppression in aged animals.

2182 EFFECTS OF SAUROBUS ANDROGYNUS ON MOUSE LYMPHOCYTES AND MACROPHAGES.

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Aims: To investigate effects of Sauropus androgynus (SA) on lymphocytes and macrophages of BALB/C mice, which is one kind of herb foods frequently eaten by Chinese people. Methods: Spleen lymphocytes and peritoneal macrophages were separated from 10 BALB/C mice. Lymphocytes and SA (0, 10, 20, 40, 80, 160 and 320mg/ml) were co-incubated with stimulator of ConA or LPS for 72 h. Culture supernatant were collected for analysis of IL-2, IL-4, IL-5, TNF and IFN-γ. Results: Compared with the ConA or LPS control group, T and B lymphocytes proliferation abilities, TNF and IFN-γ levels of SA groups (10-320mg/ml) were all depressed significantly. IL-2 of 10 and 20mg/ml SA groups increased, but that of 40-320mg/ml groups decreased. Compared with the negative control, the percentage of phagocytic cells (PP) of 20-160mg/ml SA groups and the phagocytic index (PI) of 10-320mg/ml SA groups both increased significantly. Conclusion: In summary, SA can inhibit proliferation in vitro and Th1 cytokines secretion of mouse spleen lymphocytes, while it can enhance phagocytosis of mouse peritoneal macrophages.

2183 PARTICLES FROM DUTCH TRAIN UNDERGROUND INDUCE PULMONARY INFLAMMATION IN VITRO AND IN VIVO.

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Epidemiological studies have demonstrated respiratory health effects related to exposure to ambient particulate matter (PM), specifically to particles with an aerodynamic diameter of less than 10 or 2.5 μm (PM10 and PM2.5 respectively). Recently, ultrafine or nanosize particles (PM0.1) are gaining more attention as they may have a substantial contribution to adverse health effects. A potent source of PM emission in many large cities is the underground system. However, effects of PM from this source are largely unknown. This study assessed the effects of PM collected at a Dutch underground train station. Murine macrophages (RAW 264.7) and 357BL6 macrophages, respectively, were exposed for 16 hours to 0.25-100 μg/ml and 1.0-50 μg/mouse underground PM (in vitro all size fractions, in vivo PM2.5 only). Reference materials were lipopolysaccharide (LPS) or carbon black (CB). We analyzed cell viability and protein expression of pro-inflammatory cytokines in vitro. In vivo, we determined cellular differentiation, protein expression of heme oxygenase-1 and B lymphoblast from bronchoalveolar lavage fluid (BALF), mRNA of cytokines in lung tissue as well as cytokines in blood. Underground PM induces a dose- and particle size dependent increase in TNF in RAW 264.7 cells (PM0.1> PM10> PM2.5) at nontoxicototoxic concentrations. In vivo, similar effects are observed for TNF and MIP-2 in BALF and are accompanied by an increased neutrophil cellularity (71 +/- 7.8% vs 1.0 +/- 1.3% for vehicle-treated animals). Cytokine levels in blood are all below detection limit, with exception of MIP-2 (dose-dependent increase). Underground PM did not affect mRNA expression of IL-10, IL-1β, IL-6, MIP-2 or TNF in vitro. Inherent, present data indicate that underground PM leads to a pulmonary proinflammatory response in vitro and in vivo. Our in vitro data suggest that ultrafine particles (PM0.1) are more potent than fine (PM2.5) or coarse (PM10) particles.

2184 KLH ANTIBODY RESPONSE, LYMPH NODE CELL SUBSET ANALYSIS, AND LYMPHOID TISSUE HISTOLOGY IN RATS TREATED WITH CYCLOSPORINE OR HEXACHLOROBENZENE.

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The effects of cyclosporine (CSA) and the immunostimulatory agent hexachlorobenzene (HCB) have been tested on the KLH T-cell dependent antibody (TDAB) response of rats and compared with lymphocyte subset analysis by flow cytometry as well as standard histology and immunohistochemistry of lymphoid tissues. Female SD rats were treated daily with olive oil, 20 mg/kg/day CSA or 100 mg/kg/day HCB by gavage for 32 days. KLH (0.3 mg) was injected IV on days 14 and 28, and blood samples were taken on days 1, 14 and 33 for determination of anti-KLH IgM and IgG levels by ELISA. Anti-KLH IgM and IgG levels remained below the quantification levels on all occasions in CSA-treated rats. In contrast, IgM levels were 39% higher on day 19, and IgG levels 300% higher on day 33 in HCB-treated rats than in control rats. Lymph node suspensions from CSA-treated rats showed a 51% decrease in B-lymphocytes when compared with controls, and a 28–35% decrease in T-, CD4+ and CD8+ lymphocytes. Similar, but slighter effects were seen in HCB-treated rats. Thymus weight was decreased in both CSA- and HCB-treated rats, while increases in spleen and pooled lymph node weight were noticed only in HCB-treated rats. Histological and immunohistochemical exami- nations showed B- and T-cell depletion in the spleen, lymph nodes and Peyer's patches of CSA-treated rats, and increased thymic cortex/medulla ratio. HCB in- duced marked B- and T-cell increases in the spleen and poplital lymph nodes, and decreased thymic cortex/medulla ratio. Standard hematologic changes correlated with the above-mentioned findings with either tested compound. This study pro- vides further evidence of the value of KLH TDAB in immune function assessment during rat toxicity studies.

2185 EFFECTS OF SUBCHRONIC EXPOSURE OF MUNITION RDX ENVIRONMENTAL DEGRADATION PRODUCT MNX.

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Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) is an environmental degra- dation product of high energetic munition compound hexahydro-1,3,5-trinitro- 1,3,5-triazine (RDX). Human occupational and accidental exposures to RDX and acute oral exposures in rats resulted in seizures. Earlier studies in our laboratory have reported hematotoxicity associated with acute exposure to MNX, but little is known about sub-chronic exposures of MNX. The main objective of the present study was to determine the hematotoxic effects of sub-chronic exposure to MNX in female Sprague Dawley rats. Rats were gavaged once daily for 4 or 6 weeks at a dose of 0, 25, 50, 100 or 200 mg/kg/d MNX (1/4 LD50) and sacrificed 24 h after the last dose. Toxicological endpoints measured included clinical observations, body weights, hematology, clinical chemistry, organ weights, and tissue histopathology. The major toxicological effects observed were granulocytosis and thrombocytosis in blood. Clinical chemistry indicated an increase in potassium, decrease in sodium, chloride,
glucose, and creatinine levels, and no change in albumin or total protein in serum. We also found a significant increase in relative liver weights in both 4 and 6 weeks MNX treatments with no effect on body weight gain. Bone pathology exhibited increased megakaryocytes, but fibrosis was not evident as determined by Grocott methenamine silver stain for reticulin fibers. Observed serum Na, K, Cl, glucose and creatinine changes in the absence of loss in body weight gain and serum proteins are consistent with gastrointestinal toxicity. Proliferation of megakaryocytes in bone marrow and thrombocytosis after sub-chronic MNX exposures are likely to be related mechanistically to our earlier observation of MNX hematotoxicity. (Support: United States Department of Defense).

2186 COMPARISON OF LOCAL REACTOGENICITY IN NZW RABBITS FOLLOWING MULTIPLE INOCULATIONS WITH AN ALPHAVIRUS REPLICON PARTICLE VACCINE FOR INFLUENZA FORMULATED IN HUMAN SERUM ALBUMIN OR RABBIT SERUM ALBUMIN.

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A toxicity study in NZW rabbits was conducted to evaluate systemic toxicity, immunogenicity, and local reactogenicity with a bivalent, alphavirus-like replicon particle (VRP) vaccine expressing HA and NA proteins of influenza A. This study was also designed to address the hypothesis that vasculitis observed in previous VRP toxicity studies was due to the presence of human serum albumin (HSA) in the formulation buffer. The Flu VRP vaccine, formulated in buffer containing either HSA or rabbit serum albumin (RSA), was administered on Day 1, 15, 29 and 43 by IM or SC injection. Parameters evaluated included physical examination, body weight, dermal Draize, food consumption, body temperature, clinical pathology, hematology, and macro- and micro-pathology. Dermal Draize findings were slightly more frequent in animals that received the SC Flu VRP in HSA. Total serum protein and globulin concentration and A/G ratios were altered in all immunized groups due to results of polyclonal immunoglobulin synthesis induced by vaccine administration. Pathology findings were limited to SC and IM injection sites (gross: discoloration and microscopic: degeneration/necrosis) and draining lymph nodes (axillary and iliac) and mainly in animals that received HSA in the formulation. Most importantly, vasculitis was only observed at the injection site of animals that received the Flu VRP containing HSA. The finding was resolved by Day 57/58, but was not seen in the animals that received RSA in the formulation. Anti HSA antibodies were only detectable in animals that received Flu VRP formulated in RSA. The lesions associated with Flu VRP in HSA were greater in severity grade and incidence than those associated with Flu VRP in RSA. This study supports the hypothesis that the vasculitis seen in this and previous rabbit studies was a manifestation of an Arthus-type reaction caused by the foreign protein HSA in the formulation buffer.

2187 INVESTIGATION OF BLOOD-BRAIN BARRIER (BBB) PERMEABILITY AND IMMUNE-CELL POPULATION OF BRAIN TISSUE IN BELATACEPT-TREATED MONKEYS.


A 1-month study was conducted to investigate the ability of belatacept, a selective costimulation blocker of the CD28 T-cell pathway in development for transplantation, to cross the BBB and to evaluate its effects on the presence of immune cells (antigen-presenting, T, B, and natural killer cells) and expression of CD80 or CD86 in the brain. Male cynomolgus monkeys (3/group) received 5 weekly IV doses of belatacept at 0 (saline control), 10, or 50 mg/kg (AUC [0-168h] 15,200 and 72,400 µg/h/mL, ~1X and 6X efficacious clinical exposures, respectively). Scheduled necropsies, which included whole-body saline perfusions, were conducted 24 hours after the last dose. Minute levels of belatacept (<0.07% systemic exposures) were not altered, these data collectively demonstrated that belatacept does not pass the BBB and has no effect on the presence of immune cells in the brain following 1 month of treatment in monkeys.

2188 NOSE-ONLY EXPOSURE TO JET FUEL KEROSENE DOES NOT ALTER IMMUNOCOMPETENCE IN FEMALE B6C3F1 MICE OR SPRAGUE-DAWLEY RATS.

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The purpose of these studies was to evaluate the immunotoxicology of unadditized jet fuel kerosene administered by nose-only inhalation to female B6C3F1 mice and C3H/CD Sprague-Dawley (SD) rats. The studies were conducted under Federal Good Laboratory Practices (GLP) regulations and EPA's testing harmonized guidelines for immunotoxicity (Series 870/7800). Characterization of the exposure atmosphere is provided in the accompanying abstract (DeLorme et al). 10 female C3H/CD SD rats/group were exposed to target concentrations consisting of 0 (air control), 500, 1000, and 2000 mg/m3 total jet fuel kerosene. A positive control was included for each assay. A similar design was used in the mouse study. In addition, a group of 10 unstratified animals were included to evaluate potential nose-only exposure related stress induced changes. Exposure to jet fuel kerosene in rats and mice did not produce significant effects on terminal body, spleen, or thymus weights. Overall there were no significant dose-related effects on splenic subpopulations in either species. In functional assays, no dose-related effects were observed in rats or mice in the IgM plaque assay. There was no effect in mice in the natural killer (NK) cells assay. The rat NK assay was judged unusable since response in the vehicle control animals were below historical range. No statistically significant effect was observed in either rats or mice in the delayed type hypersensitivity (DTH) response to Candida albicans. In all studies the positive control treatment worked, indicating an effect could have been detected if one had occurred. The only significant species difference observed was a dose-related increase in anti-CD3 mediated T-cell proliferation in rats which did not occur in mice. Nose-only exposure to well characterized atmospheres of jet fuel kerosene when evaluated under EPA guidelines in a well characterized atmosphere was not immunotoxic in either rats or mice.

2189 EFFECTS OF A COMPLEX MIXTURE OF PCBS ON IMMUNE FUNCTION IN B6C3F1 MICE.

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Polychlorinated biphenyls (PCBs) have been assessed for immunotoxicity in various studies; however, humans and wildlife are typically exposed to multiple PCBs environmentally. Therefore, the current study examined the effects of a complex mixture of more than 20 PCB congeners identified in dolphin blubber samples specific to the harbor environment in Charleston, SC. Immunotoxicity was examined in adult female B6C3F1 mice by evaluating lymphocyte proliferation, splenic and thymic immunophenotypes, and IgM production. Mice were exposed via oral gavage for 28 days to 0, 1.8, 3.6, 7.1, or 14.3 mg/kg/day. No changes in body weight or organ weight except liver weight were noted. Liver weight was significantly increased over control at the 14.3 mg/kg/day treatment. Lymphocyte proliferation was not altered by any treatment nor was numbers of splenic CD4/CD8 or MHCII+ subpopulations. Numbers of thymic CD4+/CD8−, CD4−/CD8+, and CD4+/CD8− cells were not altered, but numbers of thymic CD4−/CD8+ cells were significantly increased in the 14.3 mg/kg/day treatment group. Numbers of splenic CD19+/CD21−, CD19+/CD21+, and CD19−/CD21− cells were not altered, while numbers of CD19−/CD21+ cells were decreased at the 3.6, 7.1, and 14.3 mg/kg/day concentrations as compared to control. IgM production was suppressed compared to control at all treatment levels. Decreases in CD19+/CD21+ cells suggest possible alterations in numbers of follicular dendritic cells. Vascular toxicity was the most sensitive endpoint affected. As the lowest concentration tested resulted in decreases IgM production a NOAEL was not identified. The calculated ED50 for suppression of IgM production was 2.4 mg/kg/day. Additional, studies to determine the NOAEL and assess dendritic cell function are required.
MALATHION, LINDANE AND PIPERONYL BUTOXIDE, SINGLY OR COMBINED AS MIXTURES, INDUCE IMMUNOTOXICITY VIA INCREASED CELL DEATH IN MURINE SPLENOCYTES, IN VITRO.
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The potential of certain pesticides and pesticide mixtures to induce apoptosis in cultured immune cells was examined. Each of three insecticides of interest i.e. lindane, malathion and piperonyl butoxide was evaluated and found to be cytotoxic to murine (C57BL/6) splenocytes. This cytotoxicity was both, concentration- and time- dependent. Pesticide mixture studies were performed using the following concentrations based on minimum cytotoxicity (< LC25): 70μM, 50μM and 55μM for Lind, Mal and PBO, respectively. The alamarBlueTM cytotoxicity assay and cytologic analysis revealed that each individual pesticide and mixtures of malathion/lindane (Mal-Lind) and malathion/PBO (Mal-PBO) prompted varying levels of cytotoxicity (Mal 18.8%, Lind 20.4%, PBO 23.5%, Mal-Lind 53.6% and Mal-PBO 64.9%), with varying levels of apoptotic and necrotic cell death. The DNA Ladder Assay confirmed the presence of DNA fragments, a hallmark of apoptosis. Studies in laboratory animals provide the best means of assessing postnatal developmental toxicity of prospective pharmaceuticals intended for use in children. The goal of this study was to evaluate the development of immune responses in infant cynomolgus monkeys (4 to 6 months of age) to repeat subcutaneous administration of Keyhole Limpet Hemocyanin (KLH) on postnatal days 120, 150 and 180 through the production of anti-KLH specific antibodies (IgG and IgM measured by ELISA), to immunophenotype peripheral blood, spleen and thymus, and to measure total immunoglobulins (IgA, IgG and IgM).

Studies of laboratory animals provide the best means of assessing postnatal developmental toxicity of prospective pharmaceuticals intended for use in children. The goal of this study was to evaluate the development of immune responses in infant cynomolgus monkeys (4 to 6 months of age) to repeat subcutaneous administration of Keyhole Limpet Hemocyanin (KLH) on postnatal days 120, 150 and 180 through the production of anti-KLH specific antibodies (IgG and IgM measured by ELISA), to immunophenotype peripheral blood, spleen and thymus, and to measure total immunoglobulins (IgA, IgG and IgM). Administrations of KLH at 500 μg were well tolerated by the animals. No KLH-related responses were noted in clinical observations, body weights, hematology, total immunoglobulins, or on the populations of peripheral blood lymphocytes (T-cells, B-cells or Natural Killer cells). There was a robust and anticipated immune response demonstrated by the production of antibodies and a class switch from IgM to IgG KLH-specific antibodies. Following the second challenge with KLH, the mean IgM values increased by approximately 2-fold and the mean IgG values increased by approximately 5-fold. Immunophenotyping data in spleen and thymus showed expected immune development. In conclusion, infant cynomolgus monkeys show a robust immunological response following KLH challenge and, thus, are an essential component in assessing potential immunomodulating effects in preclinical biological testing.

AN IMMUNOLOGICAL RESPONSE ASSESSMENT TO KLH IMMUNIZATION IN INFANT CYNOMOLGUS MONKEYS.
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Studies in laboratory animals provide the best means of assessing postnatal developmental toxicity of prospective pharmaceuticals intended for use in children. The goal of this study was to evaluate the development of immune responses in infant cynomolgus monkeys (4 to 6 months of age) to repeat subcutaneous administration of Keyhole Limpet Hemocyanin (KLH) on postnatal days 120, 150 and 180 through the production of anti-KLH specific antibodies (IgG and IgM measured by ELISA), to immunophenotype peripheral blood, spleen and thymus, and to measure total immunoglobulins (IgA, IgG and IgM). Administrations of KLH at 500 μg were well tolerated by the animals. No KLH-related responses were noted in clinical observations, body weights, hematology, total immunoglobulins, or on the populations of peripheral blood lymphocytes (T-cells, B-cells or Natural Killer cells). There was a robust and anticipated immune response demonstrated by the production of antibodies and a class switch from IgM to IgG KLH-specific antibodies. Following the second challenge with KLH, the mean IgM values increased by approximately 2-fold and the mean IgG values increased by approximately 5-fold. Immunophenotyping data in spleen and thymus showed expected immune development. In conclusion, infant cynomolgus monkeys show a robust immunological response following KLH challenge and, thus, are an essential component in assessing potential immunomodulating effects in preclinical biological testing.

IDENTIFICATION OF STAGE SPECIFIC GENE MODULATION DURING EARLY THYMOCYTE DEVELOPMENT BY WHOLE GENOME PROFILING ANALYSIS FOLLOWING ARYL HYDROCARBON RECEPTOR ACTIVATION.
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The Aryl Hydrocarbon Receptor (AHR) is a basic helix-loop-helix transcription factor, implicated as an important modulator of the immune system and early thymocyte development. Previously we have shown that AHR activation by the environmental contaminant and potent AHR agonist, 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) leads to a significant decline in the percentage of S phase cells in intrathymic progenitor T-cell committed thymocytes 9-12 hours after exposure. This effect was strictly mediated by activation of the AHR in the developing lymphocytes. In order to identify early molecular targets, which could provide insight into how the AHR acts as a modulator of thymocyte development and cell cycle regulation, we performed a gene profiling experiment using RNA isolated from four intrathymic progenitor populations where the AHR was activated for 6-12 hours. This microarray analysis of AHR activation identified 108 distinct gene probes that were significantly modulated in the CD3-CD4-CD8+ triple negative (TN) 1-4 thymic progenitor stages 6 and 12 hours after TCDD exposure. While most of the genes identified have specific AHR recognition sequences, only seven genes were altered exclusively in the two T-cell committed stages of early thymocyte development (TN3 and TN4). Moreover, all seven of these genes were reduced in expression and five of these seven are associated with cell cycle regulatory processes. These seven genes represent novel targets of AHR transcriptional activity in the developing immune system and may offer insight into how the AHR regulates cell cycle regulation and early thymocyte differentiation.

EARLY LIFE EXPOSURE TO CIGARETTE SMOKE INCREASES LATER LIFE TUMOR SUSCEPTIBILITY POSSIBLY VIA EFFECTS ON T-REGULATORY CELLS.
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Epidemiological studies suggest that maternal smoking increases the incidence of some childhood cancers. Previous studies here have shown that elevated tumor incidence associated with prenatal cigarette smoke (CS) exposure is linked to decreased cytotoxic T-lymphocyte (CTL) activity and increased T-regulatory (Treg; CD25+Foxp3+) cells. To determine whether CS-induced increases in offspring tumor susceptibility and via effects on Treg, 5-week-old B6.C3F1 male mice were sacrificed 3, 10, 11, 19, and 26 d after mAb injection and splenocyte expression of CD25 and Foxp3 analyzed using flow cytometry; percentages of CD25+Foxp3+ cells remained ameliorated throughout the entire 26-d period. The remaining prenatally-exposed offspring injected with mAb and tumor cells were monitored for 40 d for effects on tumor incidence, growth rate, and time to tumor formation. Results demonstrated that depletion of offspring CD25+ cells ameliorated any CS-induced increase in tumor susceptibility, suggesting that prenatal CS exposure may act to increase offspring cancer risk via effects on Treg cells. Given that Treg cells can suppress anti-tumor CTL activity, findings from this study suggest a possible mechanism by which CS insult during fetal development can result in reduced offspring resistance to developing tumors. These data suggest that children of smoking mothers may be less able to mount an appropriate immune response to tumors, thus increasing their risk for cancer development later in life.

ARE DEVELOPMENTALLY-EXPOSED C57BL/6 MICE INSENSITIVE TO SUPPRESSION OF TDAR BY PFOA?
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Perfluorooctanoic acid (PFOA), used in fluoropolymer production, is environmentally persistent, present in human and wildlife populations worldwide, and associated with health effects in laboratory animals, including immunomodulation.
Several studies have reported suppression of T cell-dependent antibody responses (TDAR) in adult rodent models after 15 or 28 days of exposure. Additional studies have reported developmental toxicity to PFOA when exposure occurs from gestational day 1 (GD1) through GD17 and that a related compound, PFOS, suppresses TDAR in developmentally-exposed B6C3F1 male mice. However, the developmental immunotoxic effects of PFOA have not been explored in a rodent model. We exposed pregnant C57Bl/6 mice to 0, 0.5, or 1 mg PFOA/kg BW in drinking water from GD6-GD17. Litter size was equivalent across dose groups although litter weights were statistically decreased by 10% in the 1 mg/kg group relative to controls. On postnatal day 2 (PND2), litters were culled to 5 males and 3 females/dam. On PND21, male pups were culled and females were separated into IgM or IgG end point groups. On PND43, female pups were i.v. immunized with sheep red blood cells (SRBCs). Serum for evaluation of IgM titters was collected 5d later. Booster immunizations were given 14d later and serum for evaluation of IgG titters was collected 5d after the booster. Liver weights, lymphoid organ weights, and TDAR did not differ by dose. Although TDAR in adult mice is susceptible to PFOA exposure and the developing immune system is regarded as more susceptible to toxicant insult than the adult immune system, the doses and exposure scenario that we used did not elicit B6L6 mice injected with PDC. Hence, the mechanisms by which PFOA affects TDAR in adult mice are not known. It is possible that the developing immune system is not susceptible to PFOA in the same way as the adult immune system. However, given PFOA's developmental effects and immunotoxic effects in adult animals, further research evaluating the DIT of PFOA is warranted.


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An estimated 5-10 million women in the US were exposed to a synthetic estrogen, diethylstilbestrol (DES), to prevent miscarriages during pregnancy. Exposure to DES increases the risk of cervicovaginal cancers in “DES daughters” as well as increased incidence of autoimmune, allergies and certain infections, the mechanism of which remains unclear. In the current study, we investigated the effect of prenatal exposure to DES on immune functions in the fetus and neonate using a mouse model. Of note, the mother was exposed to DES from GD1 through GD17 and the fetus was exposed from GD15 to 16th. Fetal thymus showed sensitivity to a dose of DES that was 50 fold less than that known to cause thymic toxicity in the adult. Using HY-TCR transgenic mice to study positive and negative selection, we noted that DES altered the T cell selection process in the thymus. DES was also found to induce apoptosis in thymocytes through Fas and FasL interactions. This mechanism played a key role in toxicity inasmuch as, when pregnant lpr and gld mice (Fas and FasL deficient) were given DES, the fetuses from these mice were resistant to DES-induced toxicity in the thymus. The mouse Fas and FasL promoters were found to express at least one ERE motif and promoter analysis showed that DES induced activation of Fas and FasL may involve ERE. We also identified various other regulatory motifs present in Fas and FasL promoters and detected the presence of two nuclear factor kappa-B (NF-kB) motifs, one NF-AT motif, and two AP-1 motifs present in Fas promoter, and one NF-kB, and three NF-AT motifs present in Fasl. Fasl promoter that participated in DES-induced regulation of Fas and Fasl, respectively. Our research supports the “fetal basis of adult disease” concept by demonstrating profound effects of in-utero exposure to DES on the immune system during post-natal life (This work was supported in part by NIH grants R01ES09098, R01AI058300, R01DA016545, and F01AT003961).

IMMUNOPHENOTYPING OF CORD AND MATERNAL BLOOD: THE INFLUENCE OF EXPOSURE TO DIETARY TOXICANTS DURING PREGNANCY.

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During pregnancy, the fetus is exposed to dietary toxicants from the food intake of the mother. The immune system is especially sensitive during prenatal development, and in utero exposure to dietary toxicants may possibly cause immune-related diseases later in life. In the present study, we investigate if prenatal exposure to acrylamide, polychlorinated biphenyls (PCB 153 and 126) and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) affects the leukocyte phenotype distribution in cord blood. Pregnant women were recruited to the birth cohort BraMat, a sub-cohort of the Norwegian Mother and Child Cohort Study (Magnus et al., Int. J. Epidemiol. 35, 5 (2006)). After birth, cord and maternal blood samples were collected. Within 24 hours, immunophenotyping was performed on whole blood samples (90 cord and 54 maternal blood samples). The absolute number of T cells (CD3+CD4+ and CD3+CD8+), B cells (CD19+), NK cells (CD 16+CD56+) and monocytes (CD14+) were determined by flow cytometry. Maternal exposure to the dietary toxicants was estimated from a validated food frequency questionnaires filled in by the mothers at median. In preliminary regression analyses performed on the data from BraMat participants, dioxin, PCB 126 and 153 were not associated with leukocyte phenotype distribution. A positive association, however, was found between acrylamide exposure and the amount of B cells in cord blood (p = 0.03). The altered phenotype distribution in cord blood suggests that prenatal exposure to the dietary toxicant acrylamide may affect the immune system of fetuses. The present study will be expanded with participants (277 cord and 256 maternal blood samples) from another sub-cohort of the Norwegian Mother and Child Cohort Study.

AGE AND SEX INFLUENCE PRIMARY AND SECONDARY PREVENTION STRATEGIES FOR CONTROLLING ASTHMA AS MODELED IN THE MOUSE.

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Reducing allergen exposure to control asthma may prevent sensitization (primary prevention) or limit asthma symptoms in those already sensitized (secondary prevention). Because of varying clinical results observed in primary and secondary prevention studies, we hypothesized that age and gender influence the effectiveness of reducing allergen exposure to control asthma. Goal: Determine if greater reduction in allergen exposure is necessary to eliminate asthma symptoms (airway inflammation) in young animals compared to adults regardless of sex. To model primary prevention, we used a C3H/HeJ mice and male Balb/c mice were exposed postnatal day 19 (Young) or 82 (Adult) to decreasing doses of ovalbumin + alum (OVA; 50-0.005 mg/kg) or saline ip. On days 14-16 after sensitization, intranasal exposure to 5 mg/kg OVA elicited asthma symptoms evaluated on day 17. To model secondary prevention, mice were sensitized with 5 mg/kg OVA + alum and asthma symptoms elicited by decreasing intranasal OVA (5-0.005 mg/kg) challenge. Eosinophil peroxidase in homogenized lung was used to measure eosinophilia. For primary prevention, OVA specific IgG1 antibody did not consistently parallel changes in eosinophilia, and a ten fold greater reduction in OVA exposure at sensitization was required to prevent airway inflammation in females vs males. In secondary prevention in Young animals, a ten fold greater reduction in OVA dose eliciting symptoms was required to prevent eosinophilia in females compared to males. However, for male or female Adult animals, the same reduction in OVA challenge dose prevented eosinophilia. These data suggest that secondary prevention in young animals is more effective at limiting asthma symptoms in young compared to adults, and primary prevention is more effective for limiting asthma symptoms in males compared to females. Both gender and age must be considered when evaluating the effectiveness of allergen reduction in the home or workplace for asthma control. (Support: DOD DAMD 17-02-1-0191).

BLOOD-BASED GENOMIC PROFILES AS BIOMARKERS OF EXPOSURE AND EFFECT.

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Over the past decade, advances in genomic technology have transitioned its application from a specialized research tool to a robust, off-the-shelf commodity for both research and clinical use. Current technology has become more reliable and reproducible with broad-coverage of genomic analytes thereby providing an ideal platform for biomarker discovery, validation, and application. However, identifying genomic-based biomarkers of chemical exposure and, in particular, biomarkers of effect from most target tissues (e.g., liver, kidney) would require biopsy samples from human subjects that would be difficult, if not impossible to obtain. As a result, recent research has focused on deriving blood-based genomic biomarkers that can predict exposure and organ-specific toxicity. This session will provide an overview of the new research on the identification and validation of genomic biomarkers from peripheral blood mononuclear cells, exosomal microparticles, and circulating miRNA and mRNA with application to predicting chemical exposure and effects. This session should be of wide-ranging interest to those involved in drug development, biomonitoring interpretation, and risk assessment.
Drug-induced liver injury (DILI) is the leading cause of acute liver failure in patients and the most frequent cause of safety-related regulatory actions on drugs in the United States. Currently, there is no test that indicates DILI with certainty and the diagnosis is primarily through the exclusion of other causes. Previous research has demonstrated that cellular release of microRNAs containing mRNA into the circulation is a common phenomenon. In addition, data from trauma patients have also shown that mRNA is released by tissues into circulation post-injury. To assess whether free or microparticle-associated mRNA in circulation could be used as a biomarker for liver injury, rat models of D(+)-Galactosamine (DGAL) and acetaminophen (APAP)-induced hepatotoxicity were used. Analysis of plasma mRNA by quantitative real-time PCR (qPCR) revealed significant treatment-related increases in liver-specific albumin, haptoglobin and fibrinogen RNAs after DGAL and APAP administration, but no changes following treatment with the nonhepatotoxic bufvacaine HCL, a skeletal muscle toxicant. Serum ALT/AST levels were increased following all treatments, demonstrating a greater specificity of circulating RNAs over serum ALT/AST levels in the diagnosis of DILI. Analysis using receiver operating characteristic curves suggest that liver mRNAs in plasma are viable biomarkers for DILI. Isolation and sucrose gradient fractionation of plasma microparticles identified both microparticle-associated liver mRNA and liver mRNA associated with cellular debris. Further, plasma microparticle transcriptomic profiling following DGAL and APAP treatment revealed the generation of drug-specific profiles following DILI. Increases in circulating liver-specific RNAs and the generation of drug-specific transcriptomic profiles following hepatotoxicity indicate their potential as biomarkers that could be exploited not only in the diagnosis but also in the management of DILI.
exposure between TCDD-exposed laboratory rodent models and human populations. In order to clarify the role of hAHR, we developed a transgenic mouse that expresses hAHR specifically in hepatocytes, which have been shown to be the main cells involved in TCDD-induced liver toxicity. Using this hAHR mouse, we have demonstrated that, despite the higher relative affinity the mAHR has for specific AHR ligands, the hAHR has a higher relative ligand binding affinity for atypical ligands like indirubin. Using microarray analysis, the hAHR, compared to the mAHR, was shown to regulate a unique subset of genes in response to TCDD, some of which are involved in immune function (IL-10, MI6) and cell proliferation (TGF-alpha, EGF). These studies suggest that the hAHR may be evolutionarily adapted for binding to a distinct subset of ligands and may regulate a unique cohort of genes in response to ligand activation, compared to the mAHR in the liver. Also, these results pinpoint new inadequacies of extrapolating data obtained in rodents to define human TCDD toxicity risk. Future research conducted in mice globally expressing hAHR is needed in order to further understand the role of the hAHR in TCDD-mediated toxicity in non-hepatic tissues.

**2205** NUCLEAR RECEPTOR (CAR/PXR) HUMANIZED MOUSE MODELS TO INVESTIGATE NONGENOTOXIC HEPATOCARCINOGENESIS.

C. G. Flossbach, C. R. Flossbach, Dundee, United Kingdom.

In mice and rats, a variety of nongenotoxic chemicals activate the Constitutive Androstane Receptor (CAR) and the Pregnane X Receptor (PXR) leading to hepatic alterations (hypertrophy and hyperplasia) and, following long-term treatment, hepatocellular tumours (probably due to increased cell proliferation). Phenobarbital (PB) is representative of these chemicals and CAR activation is essential for PB-mediated hepatomegaly. The relevance of these tumours to human health is controversial due to the lack of a clear molecular mechanism and suitable human-like models. These issues were addressed using mice humanized for CAR and PXR. PB (80mg/kg ip., 4 days) was administered to double “humanized” CAR and PXR [huPXR/huCAR] mice and wild type C57BL/6J mice to investigate whether hyperplastic responses to chemicals observed in rodents are relevant to humans. Mice devoid of both receptors (PXRKO/CARKO) were used as controls. Mice were implanted with osmotic pumps containing BeD2 to replicate definitive DNA synthesis (S-phase) as a measure of cell proliferation. Liver weights were increased by PB in the wild type (WT) mice and “humanised” mice but not in the PXRKO/CARKO animals. Hepatocellular hypertrophy (histopathology) and cytomegaly (S-phase) and increased catalytic activities of Cyp2b10 and Cyp3a11 as detected by immunohistochemistry were observed in WT and huPXR/huCAR mice but not in PXRKO/CARKO mice. PB increased the hepatocellular labelling index (S-phase) approximately 5-fold in WT mice. However, no change in S-phase was detected in the huPXR/huCAR or PXRKO/CARKO mice following PB treatment. Similar results have been obtained using the nongenotoxic mouse liver carcinogen chloracne. In conclusion, these data, obtained using novel human response models, demonstrate that the human receptors are able to support the hypertrophic response but not the hyperplastic response to nongenotoxic liver carcinogens. Hence, if increased cell proliferation is essential for the tumourigenesis, it is unlikely that exposure to PB or chloracne poses a hepatocarcinogenic hazard to humans.

**2206** HUMANIZED DRUG METABOLIZING ENZYME MOUSE MODELS: POTENTIAL APPLICATION IN SAFETY ASSESSMENT OF DRUG METABOLITES.

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Nonclinical development of drug candidates may be confounded by species differences in drug metabolism. Metabolites formed in humans may be unique as compared to nonclinical test species. Metabolism in nonclinical species may also result in unique or disproportionate metabolites leading to toxicities of questionable human relevance. Genetically engineered mouse models that express human P450 enzymes could provide one potential approach to minimize the impact of metabolite-related challenges in drug development. These models may have the ability to generate major human metabolites and eliminate or reduce the formation of rodent specific metabolites. Prior to utilization of such models, it is important to qualify by characterizing protein expression, establishing whether the model generates an in vivo metabolite profile that is closely related to that of humans than the wild-type mouse, verifying genetic stability, and evaluating animal health. Since the current strategy for handling metabolite challenges through direct administration of metabolites is expensive and can significantly extend development of drug candidates, identifying an appropriate human P450 expressing model could provide a number of benefits. The benefits include improved scientific relevance of the evaluation, decreased resource needs, and a possible reduction in the number of animals used. The use of human CYP expressing mouse models may ultimately improve the quality and speed by which promising new drug candidates are developed and delivered to patients.

**2207** PPARα HUMANIZED MOUSE AND HUMAN RISK ASSESSMENT.


 Peroxisome proliferator chemicals are classic non-genotoxic carcinogens. These agents cause liver cancer when chronically administered in rodents and mice. Peroxisome proliferators include the widely prescribed lipid and cholesterol lowering fibrate drugs such as gemfibrozil and fenofibrate, used to treat patients with high serum lipids, as well as other chemicals to which humans are exposed. In contrast to the results in rodents, there is no evidence that fibrates are associated with elevated risk of liver cancer or any other neoplasms in humans thus indicating a species difference in the hepatocarcinogenic effects of these chemicals. The nuclear receptor, peroxisome proliferator-activated receptor α (PPARα), mediates the physiological response to food deprivation by altering the metabolism and transport of lipids and carbohydrates. PPARα is activated by endogenous lipid metabolites and by foreign chemicals including fibrate drugs. Genetic evidence revealed that activation of PPARα leads to the adverse biological responses such as hepatocarcinogenesis in susceptible species. However, the mechanism by which chronic activation of PPARα induces liver cancer was hitherto not established, although it is known that PPARα ligands elevate cell proliferation and inhibit apoptosis. In an effort to determine how PPARα influences hepatic toxicity and carcinogenicity, to establish the mechanism of species differences in response to PPARα ligands, and to develop a model that can be used to predict human response to PPARα ligands, PPARα-humanized mice were developed on a Ppara-null mouse background. Treatment of the PPARα-humanized mouse with the PPARα agonist Wy-14,643, led to target gene activation and lowering of serum triglycerides, but resistance to hepatocarcinogenesis. Analysis of gene expression programs and promoter occupancy of PPARα target genes in Ppara-null, wild-type and PPARα-humanized mice gave clues to target genes responsible for hepatocarcinogenesis in mice including a novel microRNA/c-myc pathway. The value of the PPARα-humanized in study of the mechanism of hepatocarcinogenesis and their use in human risk assessment will be discussed.

**2208** HUMANIZED MODELS IN THE ASSESSMENT OF NOVEL PRODUCTS USED UNDER INVESTIGATIONAL NEW DRUGS.

M. Green and J. Wally, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Rockville, MD. Sponsor: D. Bowerhof.

Humanized models are important elements in the assessment of toxicity for regulatory purposes in the Center of Biological Research and Review at FDA. Some innovative and potential promising biological products such as vaccines and others may involve toxicological pathways that are specific to humans for which the corresponding counterparts are either absent or muted in standard animal models used for regulatory safety testing. Hence the expression of toxicity using standard animal models will not provide an appropriated opportunity for the assessment of safety which is needed prior to conducting clinical studies under INDs (Investigational New Drugs) or approving new products. Humanized animal models have various strengths and weakness when used for regulatory purposes. Among the strengths are the expectations that the findings will have a greater fidelity to any potential human necessity and improve the safeguards provided to patients and subjects of IND studies. Weakness often includes a lack of historical experience to validate the model and pragmatic issues such as availability. Areas in need of humanized animal models are most critical in the assessment of potentially immunological dependent adverse effects like autoimmune mediated disease. However, other useful areas also exist as in cases where host dependent factors vary between species and are factors in adverse outcomes. Humanized animal models for toxicity assessment should be discussed in a preIND setting and prior to conducting toxicity studies which are intended to support clinical investigations. The use and selection of a humanized animal model is a developing area of experience in various regulatory settings and is subject to scrutiny and discussion by various parties. The use of humanized animal models may lead to a decrease in the number of animals used in establishing nonclinical safety information for novel products in IND clinical studies by decreasing the number of species and through an improvement in relevant physiological responsiveness.
Transcriptomics, proteomics, and metabolomics provide high-throughput global analysis of the genome and associated interactive players. These approaches are col-locally described as ‘omics technologies and have been used to enhance the un-derstanding of function, toxicological mechanisms, and risk assessment by identifying novel biomarkers of exposure including alterations in biological processes, cell signaling pathways, and specific genes. These ‘omics studies typically generate large data sets in which bioinformatic interpretation requires unique and evolving com-puter-based analytical approaches, including large databases and an assortment of analytical tools. As the largest organ of the body, the skin performs multifunctional roles as a physical barrier, physiological mediator, communicator between the exter-nal environment and internal biological processes, and a vehicle for drug delivery. The skin consists of the epidermis and dermis composed of a multitude of cell types separated by a basement membrane. The eye consists of many diverse components such as the cornea, iris, pupil, lens, retina, macula, optic nerve, choroid, and vitre-ous. The corneal epithelium functions as a barrier to the environment and is sus-ceptible to toxicant injury. The cornea must remain intact and transparent to refract light properly and disorganization of this structure can interfere with this process. The dynamic cell types of the skin and eye control multicellular processes through extensive networks of cell-to-cell communication that ultimately influence gene transcription and protein expression. Elucidating the complex cell-to-cell interactions and cellular responses to toxicants and drugs will assist in identifying biomarkers, developing safety assessment strategies, and accel-erating the development of effective medical countermeasures. The practical appli-cations of ‘omics to understanding the toxic responses will be discussed.

In the skin, monitoring gene expression profiles can be useful for enhancing the un-derstanding of dermal function, toxicological mechanisms, and risk assessment. Although there are a few transcriptomic studies in the published literature that focus on cutaneous chemical exposures, an assessment of multiple microarray data sets could be advantageous for identifying potential redundant biological mecha-nisms, signaling pathways, or genes that could guide future study direction and evaluation of dermatotoxicity, immune mechanisms, or wound healing. As in vivo cutaneous chemical exposure models can vary and the availability of raw data is often limited, extrapolations made from analyzing multiple cross-species microar-ray data sets could aid in the identification of a general set of cell signaling pathways or genes that could guide future study direction and evaluation of dermal toxico-logical assessments and potential therapeutic intervention. This presentation will re-view microarray studies in the open literature for chemical-exposed skin and focus on the potential for identifying genes and/or cellular signaling pathways that could be used as molecular therapeutic targets.

In SM toxicity. We have also utilized RNA interference to selectively inhibit signal-ing molecules to further dissect and characterize pathways that may play a role in SM-induced cell death and inflammation. Integrating the data from these large scale molecular approaches with large scale findings in histopathology and clinical data will be key to developing effective therapeutic strategies to treat SM exposure in civilian and military settings. This work was supported by the DTRA-JSTO, Medical S&T Division. The opinions are those of the authors and are not necessarily endorsed by the US Army or the Dept of Defense. The protocol was approved by the USAMRIID Animal Care and Use Committee. All proce-dures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, Publication No. 85-23, 1996), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

Over the recent years, engineered nanomaterials have been manufactured, devel-oped, and integrated into commercial products. Recently, there has been rising con-cern over what impact these materials may have on human health and the environ-ment. A tool kit combining toxicogenomics with a systems biology approach may allow screening for these effects. Primary human epidermal keratinocytes were ex-posed to several types of nano-scale materials, including single-walled carbon nan-othenus. Cultures were treated at different time points ranging from 0 to 24 h. Biotinylated RNA pellets were synthesized from total RNA isolated from the cell pellets and hybridized onto gene expression microarrays. After image analysis, the results were analyzed using a tiered approach ranging from statistical analysis to unsupervised and supervised mathematical methods. This presentation will discuss similarities and differences observed in the expression profiles between the various nanomaterial exposures, what genes were significantly affected, and which cellular pathways seemed to play a role in the exposures. Correlating these results with other studies will be discussed in terms of potential mechanisms of action.

Over the recent years, engineered nanomaterials have been manufactured, devel-oped, and integrated into commercial products. Recently, there has been rising con-cern over what impact these materials may have on human health and the environ-ment. A tool kit combining toxicogenomics with a systems biology approach may allow screening for these effects. Primary human epidermal keratinocytes were ex-posed to several types of nano-scale materials, including single-walled carbon nan-othenus. Cultures were treated at different time points ranging from 0 to 24 h. Biotinylated RNA pellets were synthesized from total RNA isolated from the cell pellets and hybridized onto gene expression microarrays. After image analysis, the results were analyzed using a tiered approach ranging from statistical analysis to unsupervised and supervised mathematical methods. This presentation will discuss similarities and differences observed in the expression profiles between the various nanomaterial exposures, what genes were significantly affected, and which cellular pathways seemed to play a role in the exposures. Correlating these results with other studies will be discussed in terms of potential mechanisms of action.

Traditional safety and toxicology has involved monitoring gross physiological and toxicological assessments and potential therapeutic intervention. This presentation will discuss similarities and differences observed in the expression profiles between the various nanomaterial exposures, what genes were significantly affected, and which cellular pathways seemed to play a role in the exposures. Correlating these results with other studies will be discussed in terms of potential mechanisms of action.
Furthermore, with increasing numbers of drug candidates and potential targets, earlier information on potential drug safety issues is required before deciding which compounds to take into the clinic. Metabolomics, the global profiling of biochemistries, provides unparalleled insight into the mechanistic action of drugs. The simultaneous analysis of hundreds of biochemistries and metabolites enables the identification of both on-target and off-target effects. Also, many of these biochemical changes are seen within hours of dosing, providing early-stage indication of drug safety issues. This presentation will demonstrate the utility of metabolomics in drug safety research through a series of case studies done in collaboration with leading pharmaceutical companies.

**2215** TOXICOLOGICAL CHALLENGES IN GREEN PRODUCT DEVELOPMENT.

E. L. Dahl. IIVS, Gaithersburg, MD.

In recent years there has been a tremendous increase in the demand for products that are green. However, this claim can be difficult to define, and in some cases (e.g., restrictions on reproductive toxins or carcinogens) conflicts with the equally desirable claim that a product was not tested on animals. A number of independent organizations have emerged with the stated goal of validating these claims to help consumers navigate a bewildering array of products in their efforts to shop conscientiously. A closer examination of the safety testing required by these certifying organizations reveals some apparent conflicts. For example, organizations offering green certification forbid carcinogens or reproductive toxins in cleaning products, while organizations offering not tested on animal claim certify hundreds of thousands of products. In setting the green standards the certifying organizations often rely on the principles of toxicology. However, standard concepts such as risk assessment are not always considered and direct involvement from experts from the field of Toxicology may be limited. Further, standard development oversight organizations such as ISO and ANSI do not specify how to balance hazard versus risk versus life cycle considerations.

**2216** THE ROLE OF TOXICOLOGY IN SETTING GREEN PRODUCT STANDARDS.


Consumers, workers and their employers are increasingly demanding green products. Products used to clean government buildings and schools are often required to be certified as green by a 3rd party. As the term “green” has no broadly recognized industry or governmental definition, companies, non-governmental organizations, and the government, it is our intention to identify strategies to reconcile apparent conflicts between green and not tested on animal claims while maintaining the high standards of safety testing required to protect human health and the environment. The overall goal is to begin building a consensus regarding a definition of green that is scientifically sound and minimizes reliance on animal testing.

**2217** DEVELOPING AND USING NON-ANIMAL TESTS FOR THE CONSUMER PRODUCTS INDUSTRY.

H. Raabe. IIVS, Gaithersburg, MD.

Assuring the safety of commercial and consumer products without testing in animals has long been the goal of many international companies. Though there can be considerable advantages to marketing products using a “not tested on animals” claim, some means of evaluating and assuring the safety of the marketed products is critical. The use of in vitro testing programs to evaluate acute skin and eye irritation is gaining widespread acceptance, and additional research activities are being conducted to address more challenging endpoints, such as allergic contact dermatitis, systemic toxicity and genotoxicity. Many individual companies have processes for adopting these technologies within their product development and safety programs. A framework for using in vitro test methods to gain market advantage, and the formal process for validating in vitro tests to assure regulatory compliance will be presented. Issues and challenges in developing, optimizing and evaluating these in vitro based programs will be discussed. The formal process for validating in vitro tests to assure regulatory compliance will be presented, with an emphasis on defining a “gold standard” to which novel tests should be compared during the validation process.

**2218** EVALUATING AND CERTIFYING GREEN CLAIMS.

C. McLellan. Toxicology Services, NSF International, Ann Arbor, MI.

Green certification forbids carcinogens or reproductive toxins in cleaning products, while organizations offering not tested on animal claims certify thousands of products. In setting the green standards the certifying organizations often rely on the principles of toxicology. However, standard concepts such as risk assessment are not always considered and direct involvement from experts from the field of Toxicology may be limited. Further, standard development oversight organizations such as ISO and ANSI do not specify how to balance hazard versus risk versus life cycle considerations. JohnsonDiversey has participated in the standard development process and will lend an industry perspective to the practice of setting green product standards and how toxicology has been used and missed in the process. Examples of controversial decisions in setting green product standards will be presented along with lessons learned and suggestions for how today’s toxicologist should approach the standard setting process.

**2219** THE U.S. EPA DESIGN FOR THE ENVIRONMENT PROGRAM.


The Design for the Environment (DfE) Program at the U.S. EPA engages a broad group of stakeholders to generate voluntary approaches to reduce use of chemicals of concern. DfE’s projects use green chemistry principles to encourage “informed substitution”, safer chemicals and transparency in our approach. Many partners do not want to engage in further animal testing to fill data gaps therefore DfE works to determine when alternatives, including computational methods and in vitro testing can be suitable substitutes. Partnerships evaluating flame retardants in furniture foam and printed circuit boards provide hazard information to inform industry in choices of chemicals when substitutions need to be made (e.g., PBDEs). Best practices in auto-refurbishing conduct workshops to train painters on efficient and safer techniques reducing toxic emissions of lead, hexavalent chromium, diisocyanates, and organic solvents. Life cycle assessments for electronic solder and computer displays identify ways to reduce释放 of toxic chemicals, analyze alternatives and identify areas for research. DfE’s projects engage stakeholders across industry, government and non-profit groups leading to tools and resources that increase sustainability and support industry interests to green their product lines.
on all new chemicals, but encourages a reduction in animal testing wherever possible. Though there are some toxicological concerns for which there are no suitable alternative testing methods, a number of non-animal testing methods work well but have yet to gain regulatory approval. Clorox is involved in several initiatives to help increase the acceptability of non-animal testing methods: 1) Develop and support partnerships that focus on the development and validation of in vitro alternative methods. This includes leveraging our knowledge from in vivo data to support validation of emerging in vitro methods. 2) Support voluntary pilot programs, such as the 18 month EPA pilot program to evaluate the use of non-animal testing for eye irritation labeling of antimicrobial cleaning products. 3) Participate along side with industry associations, government agencies, and NGO’s to develop a plan that may best assist to manage large volumes of data that will be produced as risk assessment evolves to incorporate the new paradigm proposed by the NAS Toxicity Testing in the 21st Century report. Validation and acceptance of in vitro methods by government agencies will allow faster and better-informed decision making for consumer products composed of mixtures of different chemistries.
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1. To present new developments in toxicology.
2. To provide attendees an opportunity to learn about state-of-the-art technology and how it applies to toxicological research.
3. To provide attendees an opportunity to learn about the emerging fields and how they apply to toxicology.

SESSION TYPES
Continuing Education—Emphasis on quality presentations of generally accepted, established knowledge in toxicology
Note: CE Courses will be held on Sunday.
Symposia—Cutting-edge science; new areas, concepts, or data
Workshops—State-of-the-art knowledge in toxicology
Roundtables—Controversial subjects
Historical Highlights—Review of a historical body of science that has impacted toxicology
Informational Sessions—Scientific planning or membership development
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2011 Thematic Approach
The Scientific Program Committee will continue the thematic approach for the 2011 Annual Meeting. All proposal submissions will be reviewed for their relevance under the following themes for the 2011 meeting:
• Global Air Quality and Human Health
• Novel Approaches to Preclinical Safety Assessment: Bridging the Gap between Discovery and the Clinic through Translational Toxicology
• Environment and Disease
• Toxicity Testing: State of Science and Strategies to Improve Public Health
• Integration of Toxicological and Epidemiological Evidence to Understand Human Risk
• Emerging Global Public Health Issues
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March 6–10, 2011
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